

Interface Oral Health Science 2011

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K. Sasaki, O. Suzuki, N. Takahashi

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P. Stashenko, T.E. Van Dyke,

T. Kawai, H. Hasturk, A. Kantarci

Associate Editors

Interface Oral Health Science 2011

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and the Harvard–Forsyth–Tohoku Research Workshop,
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Professor

Nobuhiro Takahashi, D.D.S., Ph.D.

Vice-Dean and Professor

Tohoku University Graduate School of Dentistry

4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

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Associate Research Investigator, Department of Periodontology

Alpdogan Kantarci, D.D.S., M.Sc., Ph.D.

Associate Member of the Staff, Department of Periodontology

The Forsyth Institute

245 First Street, Cambridge, MA 02142, USA

Editorial Liaison

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Assistant Professor

Naru Shiraishi, D.D.S., Ph.D.

Assistant Professor

Tohoku University Graduate School of Dentistry

4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

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Preface

“Interface Oral Health Science” is a new theme in dentistry, established by the Tohoku University Graduate School of Dentistry in 2002, based on the following concept: normal oral function is maintained through harmony between three biological and biomechanical systems, namely, the structure of the mouth, including teeth, the mucous membrane, bones, and muscles; microorganisms in the mouth (parasites); and biomaterials. Tooth decay, periodontal disease, temporomandibular disorder, and other oral disorders can be recognized as “interface disorders,” which are caused by the collapse of the interface between these systems. This concept is shared not only by dentistry and dental medicine but also by a variety of disciplines, including medicine, materials science, engineering, agriculture, and pharmacology.

Since 2002, the Tohoku University Graduate School of Dentistry has regarded Interface Oral Health Science as the main theme of dental research in the twenty-first century. We are committed to advancing dental research by implementing Interface Oral Health Science while promoting interdisciplinary research across a wide range of related fields. Based on this concept, we successfully organized the International Symposium for Interface Oral Health Science three times, in 2005, 2007, and 2009, to include stimulating special lectures, symposia, poster presentations, and discussions. The presentations from the three symposia were published in an English monograph series titled Interface Oral Health Science. These achievements were praised, and in 2007, “Highly Functional Interface Science: Innovation of Biomaterials with Highly Functional Interface to Host and Parasite” was adopted as a program for Research and Education Funding for an Inter-University Research Project 2007–2011, MEXT, Japan. Subsequently, we have been involved in joint research with the Institute for Materials Research, Tohoku University, and the Research Institute for Applied Mechanics, Kyushu University.

On January 6–7, 2011, as a satellite-symposium, we organized the Harvard–Forsyth–Tohoku Research Workshop at Harvard University, Cambridge, MA, USA. In that workshop, six research institutes participated, including Harvard University; the Forsyth Institute; the Institute for Materials Research, Tohoku University; the Tohoku University Graduate School of Engineering; the Research Institute for

Applied Mechanics, Kyushu University; and the Tohoku University Graduate School of Dentistry. The successful workshop comprised, precisely, interdisciplinary research outcomes across a wide range of related fields. On March 7–8, 2011, the 4th International Symposium for Interface Oral Health Science was held in Sendai, Japan, with distinguished guests invited from around the world. The gathering actually was made up of three symposia: “Bioengineered Dentistry,” “Current Activity of Research and Education in Asian Graduate Schools,” and “Highly Functional Biomaterials.” In the poster session, more than 100 posters were presented from a wide variety of fields, including “Biomechanical–Biological Interface,” “Host–Parasite Interface,” “Biomaterial Interface,” and “Social Interface.” In addition, we exchanged current knowledge and ideas about dental research and education in the future in Asia.

This book, containing the review articles from the Harvard–Forsyth–Tohoku Research Workshop and the 4th International Symposium for Interface Oral Health Science, is being published in 2011 as a special volume of the Interface Oral Health Science Series. We hope that our project, including the symposium and the book, will accelerate the progress of dental science and point the way for dental research for the future generation.

In closing, on behalf of our school I express my deepest condolences to those who suffered the devastating effects of the Tohoku Region Pacific Coast Earthquake and tsunami on March 11, 2011, especially to the victims themselves and to those who have lost loved ones. I also express my deepest appreciation for the heartfelt support and swift assistance received from colleagues all over the world.

Keiichi Sasaki

President, 4th International Symposium for Interface Oral Health Science

Dean, Tohoku University Graduate School of Dentistry

Sendai, Japan

March 2011

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Tohoku University Graduate School of Dentistry,

Minoru WAKAMORI
Hiroyuki KUMAMOTO
Satoshi FUKUMOTO
Hidetoshi SHIMAUCHI
Ken OSAKA
Takeyoshi KOSEKI
Masahiko KIKUCHI
Yasuyuki SASANO
Takashi NAKAMURA
Masahiko MARUYAMA
Mutsuo TAGUCHI
Tetsuo HAYASAKA
Yuichiro OKA
Yuki ABIKO
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Guang HONG
Risa UZUKA

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Shinichi KIKUCHI
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Hanako SUENAGA
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Naoko SATO
Yukari SHIWAKU
Takashi YOSHIDA
Ayako HASEGAWA
Junko KAWASHIMA
Azusa FUKUSHIMA
Takehiko MITO

Institute for Materials Research, Tohoku University,

Takashi GOTO

Mitsuo NIINOMI

Takayuki NARUSHIMA

Akihiko CHIBA

Kyouzuke UEDA

Eriko ITOH

Research Institute for Applied Mechanics, Kyushu University,

Yoshihiro TAKAO

Mitsugu TODO

The Forsyth Institute,

Susan COYNE

John BARTLETT

Nikos SOUKOS

Xiaozhe HAN

Susan RITTLING

Contents

Preface.	v
Acknowledgments	vii
 The 4th International Symposium for Interface Oral Health Science Symposium I: Bioengineered Dentistry	
Salivary Gland Development Has Implications for Glandular Regeneration.	3
Matthew P. Hoffman	
The Impact of Gingival Fibroblast-Derived iPS Cells in Dentistry.	9
Hiroshi Egusa	
Review: The Regulation of Tooth Development and Morphogenesis	14
Takashi Nakamura, Yoshihiko Yamada, and Satoshi Fukumoto	
Preparation and Biomedical Application of Self-Organized Honeycomb-Patterned Polymer Films	22
Takahito Kawano, Nagayoshi Iwama, Hiroshi Ishihata, Hidetoshi Shimauchi, and Masatsugu Shimomura	
 Symposium II: Current Activity in Research and Education in Asian Graduate Schools	
The Dental School We Aspire to Work for, Be Part of, and Invest Our Future in	29
Sunhun Kim	
Introducing the Metabolomics Method into Oral Science to Find Something New	31
Jinglin Zhou and Wei Li	
Eight-Year Program of Stomatology Education in China	38
Guo Chuanbin, Liu Hongwei, Jiang Yong, and Xu Tao	

Interdisciplinary and International Research/Education Based on Interface Oral Health Science	43
Nobuhiro Takahashi	
Dental Education in Mongolia	46
Amarsaikhan Bazar and Bayarchimeg Batbayar	
Symposium III: Highly Functional Biomaterials	
Design of Biomaterials for Bone Replacement Based on Parameters Determining Bone Quality	55
Takayoshi Nakano and Takuya Ishimoto	
The Development of Binary Titanium Alloys with the Aim of Dental Applications	66
Yukyo Takada, Masatoshi Takahashi, and Masafumi Kikuchi	
π-Phase and χ-Phase: New Precipitates in Biomedical Co–Cr–Mo Alloys	72
Takayuki Narushima, Shingo Mineta, Alfirano, and Kyosuke Ueda	
Session I: Biomechanical–Biological Interface	
Classification of Chewing Cycles: Different Motion Paths May Indicate Differences in Function	83
Yohei Igari, Yuko Komine, Yasue Tanaka, Mai Sato, Alexander Wirianski, and Yoshinori Hattori	
Bactericidal Effect of Hydroxyl Radical Generated by Photolysis of Hydrogen Peroxide	86
Hiroyo Ikai, Keisuke Nakamura, Midori Shirato, Taro Kanno, Atsuo Iwasawa, Yoshimi Niwano, Keiichi Sasaki, and Masahiro Kohno	
Comparison of Different Analytical Methods for Determining Singlet Oxygen Photogenerated from Rose Bengal	89
Kirika Ishiyama, Keisuke Nakamura, Hiroyo Ikai, Taro Kanno, Keiichi Sasaki, Yoshimi Niwano, and Masahiro Kohno	
Time-Specific and Stage-Specific Expression of <i>TP63</i> Gene During Osteoblast Differentiation	92
Viviane K.S. Kawata, Takashi Matsuura, Masanobu Satake, and Shuntaro Ikawa	
Metal–Allergy Cross-Reactions in Mice	95
Masayuki Kinbara, Yasuhiro Nagai, Teruko Takano-Yamamoto, Shunji Sugawara, and Yasuo Endo	
The Stomach's Response to Sham Feeding: Cephalic Vagal Effects on Postprandial Gastric Motility	97
Yuko Komine and Yoshinori Hattori	

Mechanical Properties and Degradability of HA/PLLA Composites with Different Particle Size Distribution	100
Tetsuo Takayama, Mitsugu Todo, and Hiroshi Ito	
Expression of Ten-m/Odz3 in the Fibrous Layer of Mandibular Condylar Cartilage and the Early Stage of Chondrogenic Differentiation of ATDC5 Cells	102
Nobuo Takeshita, Takashi Murakami, Tomohiro Fukunaga, Koichi Hiratsuka, Yoshimitsu Abiko, Takashi Yamashiro, and Teruko Takano-Yamamoto	
Roles of IL-6 in Mastication in Mice and Effects of Training and Food Hardness	104
Masahiro Tsuchiya, Tomomi Kiyama, Shinobu Tsuchiya, Hirohisa Takano, Eiji Nemoto, Keiichi Sasaki, Shunji Sugawara, Yasuo Endo, and Makoto Watanabe	
SDF-1 Regulation of Expression on Periodontal Ligament Cells Derived from Human Permanent Teeth	107
Takeyoshi Asakawa, Naoyuki Chosa, Tomokazu Hasegawa, Asami Asakawa, Akira Isizaki, and Mituro Tanaka	
Micro-spatial Environment and Osteoblast Osteogenesis	110
Mirei Chiba, Ryosuke Miyai, and Haruhide Hayashi	
Gene Expression in Human Osteoblasts and Periodontal Ligament Cells Under Compressive Force	112
Mirei Chiba, Ryosuke Miyai, and Haruhide Hayashi	
Establishment of Clonal Periodontal Ligament Cell Line Derived from Deciduous Tooth Immortalized by Human Telomerase Reverse Transcriptase (<i>hTERT</i>) Gene Transfer	114
Tomokazu Hasegawa, Naoyuki Chosa, Takeyoshi Asakawa, Yoshitaka Yoshimura, Akira Ishisaki, and Mitsuro Tanaka	
Pulpal Blood Flow Measurement Using a Laser Doppler Flowmetry Modified for Very Slow Blood Flow Velocity	117
Motohide Ikawa, Xiaofu Qu, Hideji Komatsu, and Hidetoshi Shimauchi	
Characterization of STRO-1 Positive Cells Derived from Human Wisdom Tooth Germs	119
Yoko Iwamatsu-Kobayashi, Daisuke Nishihara, Naoki Endo, Koji Kindaichi, Junko Kindaichi, and Masashi Komatsu	
The Influence of Mesenchymal Stem Cell Growth Medium for Isolation of STRO-1 Positive Cells from Human Periodontal Ligament	122
Naoki Endo, Yoko Iwamatsu-Kobayashi, Daisuke Nishihara, Masaaki Iwamatsu, Masashi Komatsu, and Masahiko Kikuchi	

IL-12-Mediated and IL-18-Mediated Nitric Oxide-Induced Apoptosis of Adherent Bone Marrow Cells in TNF-α-Induced Osteoclast Formation	125
Hideki Kitaura, Tomo Aonuma, Emiko Fukumoto, Keisuke Kimura, Toshiya Fujii, Zaki Weli Hakami, and Teruko Takano-Yamamoto	
Measurement of Tissue Oxygenation Level in Human Lip	128
Hideji Komatsu, Motohide Ikawa, Keishiro Karita, and Satoshi Fukumoto	
Effects of the Small Molecule Dorsomorphin on Intracellular Signaling	131
Tada-aki Kudo, Hiroyasu Kanetaka, Kazutoshi Mizuno, Yasuhiro Ryu, Ye Zhang, Mitsuhiro Kano, Yoshinaka Shimizu, and Haruhide Hayashi	
Expression of Aryl Hydrocarbon Receptor in Bone Tissues	134
Yasuhiro Miki, Shuko Hata, Ryoko Saito, Katsuhiko Ono, Hironobu Sasano, and Hiroyuki Kumamoto	
Deformation Distribution Analysis of Alveolar Bone Model in the Vicinity of a Dental Implant Using a Digital Image Correlation Method	137
Yasuyuki Morita, Mitsugu Todo, Yasuyuki Matsushita, and Kiyoshi Koyano	
Effects of Capsaicin Treatment on Nociception and Structure of Trigeminal Nerve Fibers in Adult Rats	140
Akiko Kato, Megumi Nakamura, Seishi Echigo, and Yasuyuki Sasano	
Extracellular Phosphate Increases Bone Morphogenetic Protein-2 Expression in Human Dental Pulp Cells and Human Periodontal Ligament Cells	143
Eiji Nemoto, Hiroyuki Tada, and Hidetoshi Shimauchi	
In Vitro Adherence of <i>Candida albicans</i> to Acrylic Resin with Different Surface Status	145
Taro Nomura, Tetsuya Suzuki, Junichi Furuya, Yu Shimoyama, Minoru Sasaki, and Shigenobu Kimura	
Root Elongation and Periodontal Tissue Formation in Tooth Germs Allogeneically Transplanted Between Rat Littermates	147
Koji Andou, Megumi Nakamura, Yoshinori Ina, Keiichi Sasaki, and Yasuyuki Sasano	
Booster Effect of Thermal Energy on Bactericidal Action of Hydroxyl Radical Generated by Photolysis of H₂O₂	150
Midori Shirato, Hiroyo Ikai, Keisuke Nakamura, Eisei Hayashi, Taro Kanno, Keiichi Sasaki, Masahiro Kohno, and Yoshimi Niwano	

Epigallocatechin Gallate Disturbs Thymidine Glycol Formation Induced by Oxidative Stress in Sjögren's Syndrome-Like Autoimmune Sialadenitis of MRL/lpr Mice	153
Keiichi Saito, Shiro Mori, Masao Ono, and Takeyoshi Koseki	
The Influence of Mastication on the Dopaminergic System in the Rat Brain	156
Hiroki Takahashi, Yoshihito Funaki, Akito Tsuboi, Makoto Watanabe, and Satoshi Yamaguchi	
Functional Micro-organization of the Macaque Postcentral Somatosensory Cortex Representing Orofacial Structures	159
Takashi Toda and Haruhide Hayashi	
Morroniside Derivative Regulates E-Selectin Expression in Human Endothelial Cells	161
Naomi Tanigawa, Yoshinori Takeda, Fortunatus Sunghwa, Masayuki Ninomiya, Makoto Hagiwara, Mamoru Koketsu, and Kenji Matsushita	
Regulation of Osteoblastic Differentiation by the Ubiquitin-Proteasome Pathway	164
Maki Uyama, Mari Sato, Masamitsu Kawanami, and Masato Tamura	
Downregulation of Dullard Expression Enhances BMP-Dependent Neuritogenesis in PC12 Cells	167
Ye Zhang, Tada-aki Kudo, Yoshinaka Shimizu, Mitsuhiro Kano, Yuya Sano, and Hiroyasu Kanetaka	
Session II: Host-Parasite Interface	
Inhibition of T-Cell-Mediated and Infection-Induced Periodontal Bone Resorption by TACE Blockade	173
Hiroyuki Kanzaki, Xiaozhe Han, Yukiko Asami, Maiko Suzuki, Toshihisa Kawai, and Martin Taubman	
Subgingival Plaque Biofilm Microflora of Elderly Subjects	176
Yuki Abiko, Takuichi Sato, Reiko Sakashita, and Nobuhiro Takahashi	
Divalent Cations Enhance Short-Time Fluoride Exposure-Induced Inhibition on Acid Production by <i>Streptococcus mutans</i>	178
Hitomi Domon-Tawaraya, Kazuko Nakajo, Jumpei Washio, Satoshi Fukumoto, and Nobuhiro Takahashi	
Silent Aspiration of Oral Bacteria in Elderly Patients	181
Ayako Hasegawa, Takuichi Sato, Yasushi Hoshikawa, Takashi Kondo, and Nobuhiro Takahashi	

Priming Effect of Vitamin D₃ Analog on Human Monocytic Cells in Response to Microbe-Related Ligands, Especially NOD2 Agonistic Muramyl dipeptide	183
Tomoko Ikeuchi, Takashi Nakamura, Satoshi Fukumoto, and Haruhiko Takada	
A Method for Quantitatively Evaluating Plaque Biofilm-Removing Capacity of a Dental Water Jet Using EPMA	186
Kazuo Kato, Kiyomi Tamura, Haruo Nakagaki, Shoichi Sakakibara, Youki Ou, Susumu Matsumoto, Kana Fujita, and Takuichi Sato	
Bicarbonate Decreases Inhibitory Effects of Fluoride on Acid Production by <i>Actinomyces naeslundii</i>	189
Junko Kawashima, Kazuko Nakajo, Jumpei Washio, Hidetoshi Shimauchi, and Nobuhiro Takahashi	
A New Method to Evaluate pH at the Interface Between Parasites and Biomaterials	192
Gen Mayanagi, Koei Igarashi, Jumpei Washio, Kazuko Nakajo, Hitomi Domon-Tawaraya, and Nobuhiro Takahashi	
Acid Tolerance and Endogenous Acid Production by Oral bifidobacteria	195
Kazuko Nakajo, David Beighton, and Nobuhiro Takahashi	
Maternal Transmission of Mutans and Other Oral Streptococcal Species	198
Asami Otake-Asakawa, Rikako Harada-Oikawa, Yuko Ohara-Nemoto, Mitsuro Tanaka, and Shigenobu Kimura	
A Highly Sensitive alamarBlue® Method for Evaluating Bacterial Adhesion to Biomaterials	201
Yoko Sakuma, Jumpei Washio, Yasuhisa Takeuchi, Keiichi Sasaki, and Nobuhiro Takahashi	
Fibronectin Binding Activity of <i>Streptococcus anginosus</i> Promotes Adherence to Mucosal Epithelial Cells	204
Minoru Sasaki, Yoshitoyo Kodama, Yu Shimoyama, Taichi Ishikawa, and Shigenobu Kimura	
Rapid Identification of <i>Abiotrophia/Granulicatella</i> Species by 16S rRNA-Based PCR and RFLP	206
Yu Shimoyama, Minoru Sasaki, Yuko Ohara-Nemoto, Takayuki K. Nemoto, Taichi Ishikawa, and Shigenobu Kimura	
Quantification and Identification of Bacteria in Maxillary Obturator-prostheses	209
Yasuhisa Takeuchi, Kazuko Nakajo, Takuichi Sato, Yoko Sakuma, Shigeto Koyama, Keiichi Sasaki, and Nobuhiro Takahashi	

Breath Acetone in Type 1 and Type 2 Diabetes Mellitus	212
Naoko Tanda, Yoshinori Hinokio, Jumpei Washio, Nobuhiro Takahashi, and Takeyoshi Koseki	
Combination of Mutanase and Dextranase Effectively Suppressed Formation of Insoluble Glucan Biofilm by Cariogenic Streptococci	215
Hideaki Tsumori, Atsunari Shimamura, Yutaka Sakurai, and Kazuo Yamakami	
Metabolomics of Oral Plaque Biofilm Using CE-TOFMS	218
Jumpei Washio, Gen Mayanagi, and Nobuhiro Takahashi	
Change in Infected Root Canal Microflora During the Course of Root Canal Therapy	221
Keiko Yamaki, Takuichi Sato, Ayako Hasegawa, Yuki Abiko, Kazuhiro Hashimoto, Yasuhisa Takeuchi, Junko Matsuyama, Hidetoshi Shimauchi, and Nobuhiro Takahashi	
Measuring Flow Rates of Saliva for Accurate Diagnosis of Xerostomia	223
Eiko Yoshida, Jun Suzuki, Emi Ito, Ryoichi Hosokawa, Naoko Tanda, Tohru Tamahara, Jun Harako, Katuhiko Taura, and Takeyoshi Koseki	
Session III: Biomaterial Interface	
Biomechanical Assessment for Fully Edentulous Maxilla with Dental Implants	229
Takaaki Arahira, Mitsugu Todo, Yasuyuki Matsushita, and Kiyoshi Koyano	
Morphology and Differentiation of Human Periodontal Ligament Cells on Honeycomb Films	232
Nagayoshi Iwama, Takahito Kawano, Hiroshi Ishihata, Hidetoshi Shimauchi, and Masatsugu Shimomura	
Antagonism Between Bisphosphonates (BPs) and Structurally Related Substances in the Effects of BPs in Mice	235
Satoru Okada, Tomomi Kiyama, Takefumi Oizumi, Kouji Yamaguchi, Hiroshi Kawamura, Shunji Sugawara, and Yasuo Endo	
Synthesis of Octacalcium Phosphate with Fluoride Ions and Protein Adsorption onto the Crystals	237
Yukari Shiwaku, Takahisa Anada, Yoshitomo Honda, Shinji Morimoto, Keiichi Sasaki, and Osamu Suzuki	

Corrosion Resistance of Dental Metals to Hydroxyl Radical Generated by Photolysis of H_2O_2	240
Yasutomo Yamada, Keisuke Nakamura, Yukyo Takada, Takayuki Mokudai, Hiroyo Ikai, Ryoichi Inagaki, Taro Kanno, Keiichi Sasaki, Yoshimi Niwano, and Masahiro Kohno	
Accelerated Bone Formation Around Titanium Dental Implants with Amorphous Calcium Phosphate Coating in Rabbits	243
Sou Yokota, Jun Kurihara, Naruhiko Nishiwaki, Soichiro Tamate, Kyosuke Ueda, Takayuki Narushima, and Hiroshi Kawamura	
Hydroxyapatite Film for Dental Treatment by Powder Jet Deposition: A Review.	246
Ryo Akatsuka, Hiroshi Ishihata, Miyoko Noji, Ken Matsumura, Takahiro Anada, Tunemoto Kuriyagawa, Osamu Suzuki, and Keiichi Sasaki	
Growth Behavior of Mesenchymal Stem Cell in 3D Porous Collagen Scaffolds	249
Takaaki Arahira, Mitsugu Todo, and Gouping Chen	
The Surface Wettability of Trial Acrylic Denture Base Resins	252
Maimaitishawuti Dilinuer, Guang Hong, WeiQi Wang, Taizo Hamada, and Keiichi Sasaki	
Microstructural and Mechanical Characterization of the Porous Anodic TiO_2 Layer on Titanium.	255
Zhao-Xiang Chen, Wen-Xue Wang, Yoshihiro Takao, Terutake Matsubara, and Li-Mei Ren	
Improvement of the Dentin Bond System by Reducing the Oxygen Inhibition Effect on Curing of Resin-Based Bond Materials.	257
Tatsuo Endo, Tamami Hoshino, Hiromi Sasazaki, Etsuko Miura, and Masashi Komatsu	
Curing Depth of New Sister Resin Composites of High and Low Consistency	260
Tamami Hoshino, Masafumi Kanehira, Werner J. Finger, and Masashi Komatsu	
Finite Element Analysis to Determine the Stress Distribution in Implant Components.	263
Ryoichi Inagaki and Masanobu Yoda	
Evaluation of the Accuracy of CAD/CAM Crowns Fabricated Using CT Images	265
Hiroaki Katoh, Shin Kasahara, Yoshinori Ebihara, and Kazuo Kikuchi	

Effect of Zirconia Frame Thickness on Fracture Toughness of Veneer Porcelain	268
Momoko Kudo, Shoko Miura, Masafumi Kikuchi, Ryoichi Inagaki, Joonho Cho, Keiichi Sasaki, and Masanobu Yoda	
Long-Term Clinical Evaluation of Cervical Composite Resin Restorations Treated with the Self-Etch Bonding System	271
Hiromi Sasazaki, Hideki Sato, Tatsuo Endo, and Masashi Komatsu	
Evaluation of Metal Allergies Using the Patch Test in Patients with Skin or Oral Mucosal Diseases.	273
Hideki Sato, Hiromi Sasazaki, and Masashi Komatsu	
Sterilization Effect in Low-Pressure Discharge Plasma Using Non-toxic Gas	275
Kaoru Tamazawa, Yoshinori Tamazawa, and Hidetoshi Shimauchi	
Tarnish Resistance of Experimental Ti–Ag Alloys in 0.1% Na₂S Solution	278
Masatoshi Takahashi, Masafumi Kikuchi, and Yukyo Takada	
The Development of a Dental Unit Without a Water Supply Pipe to Prevent Water Contamination	280
Yoshinori Tamazawa and Kaoru Tamazawa	
Macrophage Reaction Against Sub-micron Titanium Particles	283
Masayuki Taira, Minoru Sasaki, and Shigenobu Kimura	
The Influence of Surface Treatment of Acrylic Denture Base Resin on Peel Bond Strength Between Resilient Denture Liners	285
WeiQi Wang, Guang Hong, Maimaitishawuti Dilinuer, Taizo Hamada, and Keiichi Sasaki	
Session IV: Social Interface	
Socioeconomic Inequalities in Tooth Loss Among Japanese	291
Kanade Ito, Jun Aida, Shintaro Wakaguri, Kenji Takeuchi, Yuki Noguchi, and Ken Osaka	
Gender Differences in the Association Between Self-Rated Oral Health and Socioeconomic Status Among Japanese	294
Shintaro Wakaguri, Kanade Ito, Jun Aida, Kenji Takeuchi, and Ken Osaka	
Collaboration of Medical and Dental Facilities Promotes Oral Health Care in Hospitals	297
Jun Harako, Toru Tamahara, Ryoichi Hosokawa, Kazunori Kimura, Moritoshi Komagata, Koji Hanaoka, Seiichi Aonuma, Fusako Okuya, Kaoru Itagaki, Yukiko Akai, Kazuhiko Tamura, Hidetoshi Sonobe, and Takeyoshi Koseki	

Probing Force for Accurate Detection of Calculus on Root Surfaces	300
Emi Ito, Izumi Yuki, Ryoko Funahashi, Tomomi Ohba, Mai Takahashi, Ryoichi Hosokawa, and Takeyoshi Koseki	
Measurement of Formaldehyde Vapor Concentration of the Surrounding Air of a Dental Clinic	303
Shindoh Taku, Ikawa Kyoko, Ikawa Motohide, and Shimauchi Hidetoshi	
Model of Oral Health Care Programs to Achieve Self-Actualization in Nursing Homes	306
Miyuki Sugiyama, Jun Aida, and Takeyoshi Koseki	
Promotion of Professional Oral Care for Hospitalized and Home-Care Patients	309
Toru Tamahara, Jun Harako, Ryoichi Hosokawa, Kazunori Kimura, Moritoshi Komagata, Koji Hanaoka, Seiichi Aonuma, Fusako Okuya, Kaoru Itagaki, Yukiko Akai, Kazuhiko Tamura, Hidetoshi Sonobe, and Takeyoshi Koseki	
Relationship of Periodontal Disease and Tooth Loss to Glucose Metabolism Disorder: The Ohasama Study	312
Takashi Ohi, Yoshitada Miyoshi, Takahisa Murakami, Shiho Itabashi, Yoshinori Hattori, Akito Tsuboi, Yutaka Imai, and Makoto Watanabe	
Relationships Between Oral Health-Related Quality of Life and the Patterns of Remaining Teeth in the Middle-Aged and Elderly	315
Yoshitada Miyoshi, Takashi Ohi, Takahisa Murakami, Shiho Itabashi, Yoshinori Hattori, Akito Tsuboi, Yutaka Imai, and Makoto Watanabe	
Community Oral Health Promotion Program Fostering Self-Management for the Elderly	317
Tomoko Nishihira, Miho Nishitani, Takuichi Sato, Yuki Abiko, Kenji Matsushita, Misao Hamada, Motomi Tao, and Reiko Sakashita	
Harvard–Forsyth–Tohoku Research Workshop Tohoku University Graduate School of Dentistry	
Highly Biodegradable Bone Substitute Materials with OCP	321
Osamu Suzuki and Takahisa Anada	
Epithelial Cell Lines in the Field of Dental research: Review	327
Satoshi Fukumoto, Makiko Arakaki, Tsutomu Iwamoto, Aya Yamada, Ryoko Miyamoto, Masahiro Naruse, and Takashi Nakamura	
Metabolomic Approach to Oral Microbiota	334
Nobuhiro Takahashi, Jumpei Washio, and Gen Mayanagi	

Melastatin Transient Receptor Potential Channel Type 5	341
Minoru Wakamori, Takashi Yoshida, Takashi Kikuchi, Daisuke Kondoh, and Masashi Komatsu	
Pannexin 3, a Gap Junction Protein, Regulates Chondrocyte Differentiation in Part Through Hemichannel Activity	346
Tsutomu Iwamoto, Mariko Ono, Makiko Arakaki, Takashi Nakamura, Aya Yamada, and Satoshi Fukumoto	
Positive Effect of Whole-Body Vibration Loading on Peri-Implant Bone Healing and Implant Osseointegration	349
Toru Ogawa, Xiaolei Zhang, Ignace Naert, Keiichi Sasaki, and Joke Duyck	
Calcium Phosphate-Coated Titanium Alloy Implants Prepared by Radiofrequency Magnetron Sputtering: A Review	352
Naru Shiraishi, Yuko Suzuki, Naoko Sato, Takahisa Anada, Takashi Goto, Rong Tu, Mitsuo Niinomi, Takayuki Narushima, Kyosuke Ueda, Risa Uzuka, Osamu Suzuki, and Keiichi Sasaki	
Effects of CO₂ Laser Irradiation of the Gingiva During Tooth Movement	355
Masahiro Seiryu, Toru Deguchi, Koji Fujiyama, Yuichi Sakai, Takayoshi Daimaruya, and Teruko Takano-Yamamoto	
Regulation of Airway Responsiveness by Dopamine Receptor Signaling Pathways	359
Kentaro Mizuta	
Influence of Loading on Bone Metabolism Around Dental Implants in Rat Tibia	362
Miou Yamamoto, Masayoshi Yokoyama, Shigeto Koyama, Hiroto Sasaki, Yoshihito Funaki, Youhei Kikuchi, Hiromichi Yamazaki, Keizo Ishii, and Keiichi Sasaki	
The Forsyth Institute	
Matrix Metalloproteinase-20 and Ameloblast Cell Movement in Rows	367
John D. Bartlett	
Toll-Like Receptor Signaling in B Cell-Mediated RANKL-Dependent Periodontitis Bone Resorption	373
Xiaozhe Han, Toshihisa Kawai, and Martin A. Taubman	
Immediate Implant Stability and Function: Biomechanics and Electron Microscopy	376
Hatice Hasturk, Alpdogan Kantarci, and Thomas E. Van Dyke	

The Effect of Vitamin A on Bone Resorptive Lesions of Periradicular Periodontitis In Vivo and In Vitro	383
Jennifer Hong, Marcelo J.B. Silva, Mikihiro Kajiya, Emad Alshwaimi, Hajime Sasaki, Peter Ok, Robert R. White, Tom C. Pagonis, Bruce J. Paster, Philip Stashenko, and Toshihisa Kawai	
Immediate Implant Stability and Function: A Minipig Model and Surgical Technique	387
Hatice Hasturk, Alpdogan Kantarci, and Thomas E. Van Dyke	
Detection of RANKL and OPG in Chronic Periradicular Periodontitis	393
Peter Ok, Jun-O Jin, Mikihiro Kajiya, Christine Min, Kazuhisa Ouhara, Jennifer Hong, Robert R. White, Tom C. Pagonis, Philip Stashenko, and Toshihisa Kawai	
Role of N-Cadherin in Intercellular Adhesion, Tissue Development, Cytoskeleton Formation, and Signaling.	396
Megan L. Sierant and John D. Bartlett	
Osteopontin in the Response to Endodontic Infection	402
Susan R. Rittling	
A Potential Mechanism for the Development of Dental Fluorosis.	408
Megan L. Sierant and John D. Bartlett	
Author Index.	413
Keyword Index	418

**The 4th International Symposium
for Interface Oral Health Science**

**Symposium I
Bioengineered Dentistry**

Salivary Gland Development Has Implications for Glandular Regeneration

Matthew P. Hoffman

Abstract. The permanent loss of salivary gland secretory function often occurs after irradiation of head and neck tumors or removal of salivary gland tumors, in autoimmune diseases such as Sjögren's syndrome, and in rare genetic syndromes in which salivary gland development does not occur. The oral health of these patients is severely compromised due to the permanent loss of saliva production, which diminishes quality of life. Current therapeutic options are limited and therefore the regeneration of salivary glands becomes an important therapeutic goal. This may involve using a patient's own progenitor cells isolated from a biopsy to repair the damaged or diseased tissue. However, we have only a limited understanding of salivary gland progenitor cell biology. This paper will provide an overview of what was discussed at the Interface Oral Health Science Symposium on Bioengineered Dentistry concerning the function of progenitor cells during salivary gland development.

Key words. Keratin 5, Progenitor cell, Regeneration, Salivary gland, Stem cell

1 Introduction

An important aim in salivary gland research is to understand how multiple regulatory inputs drive development, from the earliest stages of progenitor cell commitment, maintenance, and differentiation, to growth and morphogenesis, culminating in a functional salivary gland. Understanding how progenitor cells function during embryonic salivary gland development may provide a logical basis for designing approaches to regenerate damaged adult salivary glands. The progenitor cells of

M.P. Hoffman (✉)

Matrix and Morphogenesis Section, Laboratory of Cell and Developmental Biology,
National Institute of Dental and Craniofacial Research, National Institutes of Health,
Room 433, Bldg. 30, 30 Convent Dr, MSC 4370, Bethesda, MD, USA
e-mail: mhoffman@mail.nih.gov

interest, from a regenerative perspective, would be able to develop into the secretory acinar epithelial cells of the gland. Therefore, identifying the epithelial progenitor cells of the salivary glands will enable us to investigate how to direct their differentiation into secretory cells, as well as to maintain a pool of undifferentiated progenitor cells for normal tissue homeostasis.

2 Submandibular Gland Development

The developing mouse submandibular gland has been widely used as a model to study salivary gland development. Many reviews have been published describing salivary gland development in the mouse [1, 2], and comparing it to human development [3]. Briefly, the mouse salivary gland placode initiates as a localized thickening of the oral epithelium at day 11 of embryonic development (E11). By E12, the salivary placode expands into the underlying mesenchyme and forms a primary bud on a stalk. Half a day later, at E12.5, the surrounding mesenchyme, which is derived from the migrating neural crest cells, condenses around the epithelium. This mesenchyme also contains endothelial cells and neuronal cells. The neuronal cells coalesce around the primary duct and form the parasympathetic ganglion. By E13, the epithelial end bud increases in size by proliferation and undergoes a process of cleft formation, which results in 3–5 end buds. At this time, the sublingual gland also begins to develop anterior to the developing SMG within the same condensed mesenchyme. The primary duct of the SMG elongates rapidly *in vivo* to form the Wharton's duct, and the initial end buds form the ductal architecture and body of the SMG. After E13.5, a process termed branching morphogenesis begins, with successive rounds of end bud clefting, epithelial proliferation, and secondary duct formation. After E15, the end buds polarize and secretory cell cytodifferentiation begins. After birth, the sympathetic nerves grow into the gland, extending axons along blood vessels into the gland. Postnatal salivary gland development, which involves further secretory cell differentiation and the formation of the granular convoluted tubules, has been previously described [4].

3 Progenitor Cells

Progenitor cells, which are multipotent cells, rapidly divide and differentiate into numerous cell types to form the gland during embryonic development. In the adult gland, the progenitor cells are less proliferative and are activated as part of the normal turnover of cells in the gland to replace the acinar and ductal cells and, in cases of injury, to repopulate and recreate the glandular architecture. Most reports on salivary gland progenitor cells study the adult SMG, but it is not yet known if the progenitor cells in the embryonic SMG are the same as the adult. The salivary progenitor cell populations reported in the literature include label-retaining cells (LRC)

and cells expressing markers such as integrin alpha 6, the KIT receptor, the transcription factor ASCL3, or keratin 5.

Studies using BrdU pulse-labeling have identified LRCs, which are slowly dividing progenitor cells. In these types of experiments, the cells labeled with BrdU include acinar cells, ducts, myoepithelium, and mesenchymal cells, suggesting that multiple slowly-dividing progenitor cell-types exist in SMGs [5]. In models of gland injury, such as those with reversible ductal ligation, it is the intercalated duct cells that proliferate after injury [6]. Fluorescence-activated cell sorting (FACS) isolation of progenitor cells from the adult SMGs of mice, rats, and swine have been used to obtain cells for salivary regeneration studies by many research groups. Antibodies to a variety of cell surface epitopes have been used to isolate cells that are Sca1-positive (+) and KIT+ [7], or integrin $\alpha\beta 1+$ [8], or Thy1+ and integrin $\alpha\beta 1+$ and integrin $\alpha\beta 4+$ [8, 9]. Interestingly, salivary gland progenitor cells were able to trans-differentiate into both pancreatic and hepatic cells [7, 10], and could regenerate hepatectomized livers. Thus, the salivary gland could also be a source of cells for regeneration of other tissues.

One of the most promising cell surface markers used for isolating progenitor cells from SMGs is KIT. KIT is a transmembrane cell surface receptor involved in protein tyrosine kinase signaling pathways, and it binds the growth factor Kit ligand (KITL), which regulates stem cell self-renewal in other organ systems. KIT is most widely studied for its role in hematopoiesis, however KIT+ cells isolated from mouse SMGs by FACS were transplanted into an irradiated mouse SMG and restored gland function and morphology [11].

Another marker used to identify salivary progenitor cells is ASCL3, a transcription factor. ASCL3+ cells are in the duct of the SMG and recent lineage tracing studies reported that *Ascl3*-expressing cells form acinar and ductal cells in the major salivary glands [12]. Surprisingly, genetic ablation of the *Ascl3*-expressing progenitor cell population did not affect SMG development [13]. These data are important because they demonstrate that, even with the loss of one cell subpopulation in the gland, another progenitor cell type can expand and compensate for its absence.

An important model for understanding SMG regeneration is the reversible ligation of the primary duct in adult rats or mice, which results in rapid atrophy of the gland. After removal of the ligature, the gland regenerates completely only if the parasympathetic nerves remain intact [14]. These data highlight the fact that parasympathetic function is essential for the progenitor cells to regenerate. Therefore, our laboratory investigated the role of parasympathetic innervation during SMG development to determine whether it also influences progenitor cell function during formation of the gland. We used organotypic recombination experiments to remove the parasympathetic ganglion (PSG) from embryonic SMGs in explant culture and found that epithelial branching was significantly reduced in the absence of the PSG [15]. One of the major neurotransmitters produced by the PSG is acetylcholine. Another loss-of-function approach involved inhibiting the acetylcholine muscarinic receptor 1 (M1), which is present on SMG epithelial cells. Addition of the muscarinic receptor antagonist (DAMP) decreased epithelial morphogenesis in SMG

explant culture. Analysis of gene expression in recombined SMG explants cultured with or without the PSG showed a significant reduction in several genes, including keratin 5, keratin 15, and aquaporin 3. The addition of the muscarinic receptor antagonist also resulted in a reduction in the expression of these genes within 4 h. Furthermore, FACS analysis and immunohistochemistry confirmed the decrease in the number of keratin 5 positive (+) cells and in protein expression with loss of PSG function. Importantly, keratin 5, keratin 15, and aquaporin 3 have been reported to be markers of epithelial progenitor cells in other organs, which suggested that the PSG, via acetylcholine/M1 signaling, regulated progenitor cell function during SMG development. However, we needed to confirm that the keratin 5+ cells were also progenitor cells in the mouse SMG by lineage tracing experiments. In these experiments, keratin 5-expressing cells were marked with a reporter gene at an early stage of gland development so that the cell fates of their progeny could be tracked throughout development with the reporter gene. These studies demonstrated that most of the epithelial structures of the postnatal SMG were derived from keratin 5 precursor cells. In terms of investigating PSG function, we also used gain-of-function approaches and added carbachol, a muscarinic agonist that increased epithelial branching and keratin 5 expression in the SMG epithelium. Importantly, carbachol rescued epithelial morphogenesis and keratin 5 expression in recombined PSG-free explants. Rescue of both epithelial morphogenesis and keratin 5 expression was dependent upon transactivation of the epidermal growth factor receptor (EGFR). It has been reported that muscarinic receptor activation can transactivate the EGFR [16] and, as such, addition of an EGFR antagonist blocked the effect of carbachol to increase keratin 5+ cell proliferation. It is also important to note that when the keratin 5+ cells proliferate, they can either divide to generate identical keratin 5+ cells or differentiate and begin to express keratin 19. Immunostaining experiments demonstrated that the keratin 5+ cells were more basally located in the ducts, whereas the keratin 19+ cells were located nearer to the lumen of the duct. FACS analysis demonstrated that carbachol stimulation doubled the number of keratin 5+ cells that did not express keratin 19, whereas the addition of HBEGF, which activates the EGFR, significantly increased proliferation of keratin 5+ and keratin 19+ cells. Therefore, the acetylcholine secreted by the nerves maintains the keratin 5+ progenitor cells and EGFR activation also increases ductal differentiation of these cells as evidenced by an increase in keratin 19 expression. Thus, the PSG is essential for SMG development, maintaining the keratin 5 expressing progenitor cell population through an ACh/M1/EGFR signaling pathway in the epithelium. These data have implications for postnatal epithelial regeneration, which will require muscarinic stimulation of the keratin 5+ progenitor cells. In addition, we also demonstrated that a similar mechanism occurs in adult SMGs by culturing denervated lobules of adult SMGs and treating them with carbachol and the muscarinic and EGFR antagonists. In a similar manner to the embryonic progenitor cells, muscarinic stimulation increased the expression of keratin 5, which was inhibited by the M1/EGFR inhibitors. In addition, it was recently demonstrated in the adult gland that, after ductal ligation is removed, there is an increase in the number of keratin 5+ cells during the regeneration process of the SMG [17].

4 Conclusion

An important future goal is to understand how the regulatory inputs from the PSG, growth factor signals from the mesenchyme, and signaling from M1/EGFR are integrated within keratin 5+ progenitor cells during SMG development. In addition, understanding the mechanisms by which both keratin 5+ and KIT+ progenitor cells self-renew and differentiate into different cell types in the SMG will be critical to understanding organogenesis, and will provide important information about different progenitor cell populations that could be used for regenerative therapy. It will be important to identify cell surface molecules by which progenitor cells can be FACS sorted, as well as to define the culture conditions by which progenitor cells isolated from a patient biopsy can be expanded in culture without differentiating. During treatment of a patient it will be important to direct the progenitor cell growth with factors that allow both maintenance of a progenitor cell reservoir, and differentiation of specialized cell types to repair or regenerate the damaged tissue.

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The Impact of Gingival Fibroblast-Derived iPS Cells in Dentistry

Hiroshi Egusa

Abstract. The oral gingiva, which is often resected during dental treatments, is an easily obtainable tissue, and cells can be isolated from patients with minimal discomfort. We generated induced pluripotent stem (iPS) cells from adult mouse or human gingival fibroblasts (GFs) using retroviral transduction of the Yamanaka factors (Egusa et al., PLoS One, 2010). These iPS cells exhibited characteristics similar to embryonic stem cells. The reprogramming efficiency of mouse GFs was higher than that of tail skin fibroblasts. Fibroblasts from gingival tissues that are easily obtained by dentists can be readily reprogrammed into iPS cells, thus making them a promising cell source for future oral tissue engineering applications, as well as for in vitro applications for drug screening and the generation of disease-specific iPS cells for tailor-made diagnostics. Current hurdles that will need to be cleared if iPS cells are to fulfill their clinical promise are also outlined in this mini-review.

Key words. Dentistry, Gingival fibroblast, iPS cell, Reprogramming, Tissue engineering

1 Introduction

Tissue engineering is a new frontier in dentistry with the aim of achieving jawbone and tooth regeneration [1], and engineering applications await the establishment of a stem cell source that allows for easy collection by dentists [2]. Growing evidence has demonstrated that mesenchymal stem cells are found in various tissues, and that certain tissues contain more stem cells than others. Among these tissues, dental

H. Egusa (✉)

Department of Fixed Prosthodontics, Division of Oromaxillofacial Regeneration,
Osaka University Graduate School of Dentistry, 1-8 Yamadaoka,
Suita-city, Osaka 565-0871, Japan
e-mail: egu@dent.osaka-u.ac.jp

tissues are considered a rich source of mesenchymal stem cells that are suitable for tissue engineering applications [3]. However, the application of mesenchymal stem cells for quality-controlled cell banking for regenerative medicine may be limited because their proliferation and differentiation capacities decrease after repeated culture expansion *in vitro*.

Induced pluripotent stem (iPS) cells are stem cells that have been created from adult somatic cells such as those of the skin [4, 5], liver, stomach [6], blood [7] or tissue stem cells [8, 9] through the introduction of genes (e.g., Oct3/4, Sox2, Klf4 and c-Myc) that reprogram the cells and transform them into cells that have all the characteristics of embryonic stem (ES) cells. iPS cells have a great potential for tissue-specific regenerative therapies, since they make it possible to avoid the ethical issues surrounding the use of ES cells and the problems with rejection following the implantation of non-autologous cells [10]. This mini-review describes the generation of iPS cells from the gingiva and the possible applications of iPS cells in the field of dentistry.

2 Generation of iPS Cells from Gingival Fibroblasts

The reprogramming process using the original methods for iPS cell generation [5, 11] appears to be highly inefficient, and is likely affected by many factors, including the age, type and origin of the cells used. However, iPS cells efficiently generated from accessible tissues have the potential for numerous clinical applications. The oral gingiva, which is often resected during general dental treatments and up to now been treated as biomedical waste, is an easily obtainable tissue, and cells can be isolated from patients with minimal discomfort. The gingiva is composed of a thin keratinocyte layer with underlying connective tissue. Gingival fibroblasts (GFs), which are the main constituents of the gingival connective tissue, are phenotypically different from other fibroblasts [12], and play an important role in oral wound healing [13]. Interestingly, experimental animal studies and clinical observations have consistently shown that wound healing in the oral mucosa has better outcomes than that in the skin [14, 15], although the healing processes and sequences are similar. Therefore, it has been postulated that oral mucosal cells possess distinct characteristics to accelerate wound healing [13], which might give an advantage of cell reprogramming. In addition, primary GF cultures can be easily established, because GFs adhere and spread well on culture plates, and proliferate well under relatively simple culture conditions [16].

We generated iPS cells from adult wild-type mouse GFs via introduction of the Yamanaka factors (Oct3/4, Sox2, Klf4 and c-Myc; GF-iPS-4F cells) or three factors (the same as used for GF-iPS-4F cells, but without the c-Myc oncogene; GF-iPS-3F cells) (Fig. 1a) [17]. These iPS cells exhibited the morphology and growth properties of ES cells and expressed ES cell marker genes, with a decreased CpG methylation ratio in the promoter regions of *Nanog* and *Oct3/4*. Additionally, teratoma formation assays showed the ES cell-like derivation of cells and tissues representative of

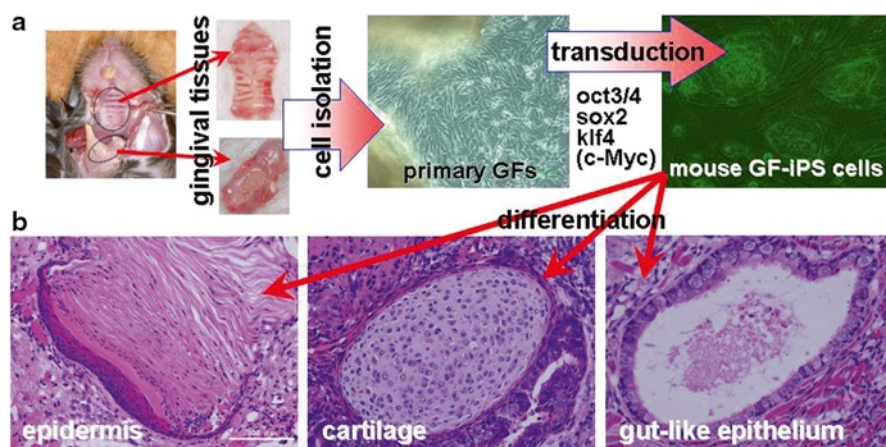


Fig. 1. (a) Generation of mouse gingival fibroblast (GF)-derived iPS cells. Gingival tissues of adult mice were extracted for establishment of primary GFs. After three- or four-factor retroviral transduction, several ES cell-like colonies emerged and some of the colonies were selected for clonal iPS cell cultures. (b) H&E staining of teratoma sections showed differentiation of mouse GF-iPS cells into various tissues from all germ layers, including ectodermal epidermis, mesodermal cartilage and endodermal gut-like epithelium. The figure was reproduced under the open-access license policies of the Public Library of Science (PLOS), taken from PLOS One (Egusa et al. [17])

all three germ layers (Fig. 1b). When transplanted into blastocysts, the GF-iPS-3F cells gave rise to chimeras and contributed to the development of the germline. Notably, the reprogramming efficiency of mouse GFs was higher than that of fibroblasts isolated from tail tips, possibly because of their higher proliferative capacity.

iPS cells were also successfully generated from human gingival fibroblasts, established from healthy gingival tissues discarded during dental implant surgery, using retroviral transduction of the Yamanaka factors. These results suggest that GFs from easily obtainable gingival tissues can be readily reprogrammed into iPS cells, thus making them a promising cell source for investigating the basis of cellular reprogramming and pluripotency for future clinical applications [17].

3 Future Directions and Problems with iPS Cell Technology in Dentistry

In the future, for both medical and dental fields, the applications of iPS cells are highly anticipated due to their usefulness in regenerative medicine, disease modeling and the development of pharmacological agents. In dentistry, gingival tissue-derived iPS cells hold great potential for dental tissue regeneration, such as for jaw bones, periodontal tissues [18], salivary glands and teeth. However, although major

progress in iPS cell research has been achieved, the applicability of these cells in clinical trials still has to be carefully investigated.

It is important to first establish xeno-free generation and culture methods for human iPS cells before such cells can be made available for clinical transplant applications. Many trials to generate and culture iPS cells in xeno-free conditions are under way, such as using virus-free [19, 20] and animal feeder cell-free methods [21, 22]. Another problem to be solved is how the direction of iPS cell differentiation toward the distinctly guided cell/tissue type can be guided to avoid tumor formation in the transplanted site. The potential use of iPS cells for tissue engineering awaits an efficient protocol that guides iPS cells to a prescribed targeted cell differentiation, while simultaneously avoiding the risk of tumor development. We are currently investigating whether iPS cells from gingival tissues can be readily guided into osteogenic cells, and whether osteogenesis can be enhanced by small molecules.

It should be noted that another basic concern regarding the clinical use of iPS cells has recently arisen. It has been generally assumed that the application of patient-specific iPS cells to replace lost or damaged cells in the body would possess major advantages, because autologous iPS cells given to the patient would be recognized as the body's own cells, and should therefore be tolerated by the immune system. However, this benefit remains theoretical for now; because Zhao et al. [23] recently reported that the abnormal gene expression in some cells differentiated from iPS cells can induce T-cell-dependent immune responses in syngeneic recipients. Prior to the clinical application of autologous iPS cells for patients, the immunogenicity of patient-specific iPS cells must be extensively evaluated.

Nonetheless, generating iPS cells from oral gingival cells represents a major step forward in developing not only a successful cell-based treatment for missing oral tissues, but also oral disease modeling and the development of pharmacological agents for alveolar bone augmentation, oral cancer treatment, and so on. Technologies using oral tissue-derived iPS cells would provide a significant advance in stem cell research in dentistry, which has the potential to open up new avenues for dental research and personalized treatment.

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Review: The Regulation of Tooth Development and Morphogenesis

Takashi Nakamura, Yoshihiko Yamada, and Satoshi Fukumoto

Abstract. A variety of vertebrate organs, including teeth, begins their development by inductive sequential and reciprocal interactions between epithelium and mesenchyme. In tooth development, the interactions between ectodermal-derived epithelium and the cranial neural crest-derived mesenchyme regulate the shape, position, and size of the tooth crown with a functional cusp. During tooth development, many signaling molecules and transcription factors regulate tooth development and morphogenesis. Recently, we reported Epiprofin, an Sp transcription factor, is expressed during tooth development and exerts critical roles in dental epithelial differentiation and the determination of tooth number. In this review, we describe the expression pattern and functions of Epiprofin in tooth development.

Key words. Epiprofin, Supernumerary teeth, Tooth development, Tooth morphogenesis

T. Nakamura (✉) and S. Fukumoto

Division of Pediatric Dentistry, Department of Oral Health and Development Sciences,
Tohoku University Graduate School of Dentistry, Sendai 980-8575, Japan
and

Laboratory of Cell and Developmental Biology, National Institute of Dental and Craniofacial
Research, National Institutes of Health, Bethesda, MD 20892, USA
e-mail: taka@dent.tohoku.ac.jp

Y. Yamada

Laboratory of Cell and Developmental Biology, National Institute of Dental and Craniofacial
Research, National Institutes of Health, Bethesda, MD 20892, USA

1 Tooth Development

The developing tooth is an excellent model to study the molecular mechanisms involved in epithelial-mesenchymal interactions. The first morphological manifestation of tooth development in mice is the formation of the dental lamina, a thickening of the oral epithelium at E11.5. Subsequently, the dental lamina grows into the underlying mesenchyme of the first branchial arch, thereby forming epithelial buds (E13.5). During the cap stage of development, the condensed dental mesenchyme diverges into two different pathways: the dental papilla that gives rise to dentin-secreting odontoblasts and dental pulp fibroblasts; and the dental follicle that contains progenitors for cementoblasts and osteoblasts, and periodontal ligament fibroblasts [1]. After the cap stage, the tooth germ progresses to bell stages, and epithelial cells differentiate into enamel-secreting ameloblasts. Dental epithelium differentiates into ameloblasts through mainly five distinct stages, i.e., (1) proliferative stage, (2) differentiation stage, (3) secretory stage, (4) early maturation stage, and (5) late maturation stage [2]. At the proliferative stage, dental epithelium proliferates rapidly. At the differentiation stage, the cells stop proliferating and differentiate into preameloblasts, which show cellular polarity and begin to secrete enamel matrix proteins. At the secretory stage following dentin mineralization, differentiated ameloblasts deposit enamel matrix proteins including amelogenin, ameloblastin, enamelin, and tuftelin, and additionally amelotin are secreted in later stages. During the maturation stage via the transitional stage when ameloblasts eventually undergo apoptosis, the enamel matrix is almost completely replaced by calcium and phosphorous, and ameloblasts eventually give rise to reduced enamel epithelium at the regressive stage [3].

2 Epiprofin

Epiprofin has been identified through the oral genome project as part of the NIDCR (National Institute of Dental and Craniofacial Research, NIH) initiative for tooth and craniofacial study. To obtain cDNA clones preferentially expressed in tooth, we differentially screened DNA microarrays containing about 12,000 clones from E19.5 molar cDNA library with fluorescently labeled probes from E19.5 molar and E13.5 body mRNA. We identified 197 cDNA clones that are preferentially hybridized to RNA probes from E19.5 molar. The majority of these clones encode enamel matrix proteins, such as ameloblastin, amelogenin, and enamelin, indicating feasibility of the microarray analysis. Finally, 12 out of the 197 clones have been found as unknown proteins or correspond to ESTs previously deposited in GenBank. We have identified a cDNA clone for Epiprofin (Epf_n) (NCBI GenBank™ accession number AY338955), which is preferentially expressed in tooth [4]. Epf_n is a new member of the Sp/KLF transcription factor family. This family consists of more than 21 proteins in humans and 17 in mice, which have a DNA-binding domain with three tandem C₂-H₂-type zinc finger motifs at the C-terminus, and transcriptional

regulatory domains at the N-terminus [5]. Sp factors comprise nine member gene family encoding transcription factors that play an essential role in regulating a wide variety of developmental and cellular processes, including cell growth, differentiation, apoptosis and tumor formation [6, 7]. Expression of *Epf*n is detected at the initiation stage of tooth development. *Epf*n is clearly expressed in dental epithelium of dental lamina and not expressed in dental mesenchyme at the early stage of tooth development. In bud stage, dental epithelial cells are rapidly proliferating to form a tooth bud. During bud stage, *Epf*n is expressed widely in dental epithelial cells. At the cap stage, dental epithelial cells determine their cell fate into several lineages such as stellate reticulum, and inner and outer enamel epithelium. At this stage, the expression of *Epf*n is limited in inner enamel epithelium and not expressed in other dental epithelial cell types. At the bell stage, *Epf*n continuously is expressed in pre-ameloblasts and ameloblasts. Interestingly odontoblasts, which derived from dental mesenchyme, start expressing *Epf*n.

Functional studies revealed that *Epf*n transcription factor promotes cell proliferation, suggesting a role in regulating cell growth during the development of tissues of ectodermal origin [4]. Over-expression of *Epf*n exerts distinct roles in cell growth by transient or stable expression in dental epithelial cells. By transient expression of *Epf*n in primary dental epithelial cells were strongly stimulated their cell mitogenic activity. It can be considered that transient expression of *Epf*n in dental epithelial cells might mimic the progenitor cell types of dental epithelium, which give rise to differentiate into ameloblasts. On the other hand, stable expression of *Epf*n inhibits cell proliferation. We also demonstrated that over-expression of *Epf*n promotes dental epithelial cell differentiation into ameloblast, which is in a terminal differentiated phase of dental epithelial cells and stop cell proliferation [4]. Continuous expression of *Epf*n could induce cell cycle exit due to the rapidly promotion of dental epithelial cell differentiation into ameloblast. More recently, an excess number of teeth, enamel deficiency, defects in tooth cusps and root formation, and abnormal dentin structure have been shown in *Epf*n knockout (*Epf*n KO) mice [8].

3 Regulators in Tooth Development

The interactions between the epithelial and mesenchymal tissues in tooth morphogenesis are mediated by growth factors and signalling molecules [9]. For instance, the role of *Fgf8* has been extensively analyzed during the initiation of tooth morphogenesis. Transcripts of *Fgf8* are expressed in early dental epithelium and the translated protein induces the expression of a number of genes in the early dental mesenchyme, which are involved in the acquisition of odontogenic competence such as *Msx1*, *Pax9*, *Activin-βA* and *Dlx1/Dlx2*. The significance of those molecules in early tooth development is demonstrated by analysing their gene targeting mouse models. For example, *Msx-1*, *Lef-1*, *Pax-9* and *Activin-βA* knockout mice, tooth development arrests at the bud stage [10–13].

Other signalling molecules in tooth development include Sonic hedgehog (Shh), which is one of the earliest marker for the dental lamina in E11 mouse embryos. During the bud stage, the expression of Shh is reduced, and is subsequently upregulated in the enamel knots at the cap stage. From the first enamel knot (E14.5) the expression spreads to the inner enamel epithelium. It has been suggested that Shh could play a role as an inhibitor regulating the distance between forming cusp [14]. In addition, Shh appears to be an important stimulator of cell proliferation, as shown earlier in other ectodermal organs including skin and hair [15]. Conditional mutant mice have showed that Shh controls the development of tooth shape by differentially regulating growth [16].

4 Regulators in Tooth Number

Hypodontia (Agenesis of one or more teeth) is currently the most common of human developmental anomalies [17–19]. Failure of one or more of the third molars to form occurs in 20% of the population. The reported incidence of teeth other than third molars being missing varies from 1.6% to 9.6% [18]. Two mutations causing isolated tooth agenesis have been identified. A point mutation in the MSXJ gene (4p16) was identified in affected members of a family with missing second premolars and third molars [20], and a mutation of PAX9 gene (14q21-ql3) was associated with oligodontia affecting most molars [21]. In addition, a recessive form of tooth agenesis has been mapped to chromosome 16 [22].

Hyperdontia, the formation of one or more supernumerary teeth occurs much less frequently than agenesis. For humans, the most frequent site for these occurrences are the maxillary central or lateral incisor regions (mesiodens). Such teeth may have a highly aberrant form, tucked-in to the lingual of the normal tooth row. They may take on the form of neighboring teeth. Supernumerary teeth often do not erupt, therefore, any survey of living or fossil individuals must necessarily include panoramic radiography to reveal unerupted teeth.

Supernumerary teeth are defined as those that are present in excess of the normal component of human dentition. The pathogenesis of extra teeth formation is poorly understood in human population. In recent years, scientists have found some advances in our understanding of the genetic basis of supernumerary teeth. Runx2 are known to be involved in the regulation of tooth number and they are thought to be transcriptional targets of FGF signaling [23, 24]. And many biological evidences provide insight into why supernumerary tooth formation may occur. Indeed, many of the molecular signaling pathways known to be involved in normal development of the tooth germ can also give rise to additional teeth if inappropriately regulated. These include components of the FGF, Wnt, TNF and BMP families, which provide a useful resource of candidate genes that may potentially play a role in human supernumerary tooth formation.

Cleidocranial dysplasia (CCD, OMIM #119600) is a skeletal disorder with autosomal dominant inheritance. The clinical hallmarks of CCD are short stature,

delayed closure of cranial fontanels and sutures, Wormian bones, frontal bossing, supernumerary and late erupting teeth, rudimentary or absent clavicles, wide pubic symphysis, and other skeletal anomalies [25]. RUNX2, located on chromosome 6p21, has been identified as a gene responsible for CCD [26, 27]. Regarding dental abnormality, CCD is associated with supernumerary teeth, and the delayed eruption and impaction of permanent teeth [28–30]. The position and number of supernumerary teeth vary among cases, but they are seen below the permanent teeth (incisors, canines, and bicuspid) that have replaced with the deciduous teeth.

In mouse model, supernumerary teeth have been reported in some mutant mouse lines including *Spry2* and *Spry4* knockout mice [31]. *Spry2* is expressed in dental epithelium, while *Spry4* is expressed in dental mesenchyme and both *Spry2* and *Spry4* act as an antagonist for FGF signaling [31]. Either *Spry2* or *Spry4* knockout mice develop the supernumerary tooth in diastema by hypersensitivity of FGF signaling in dental epithelium [31].

Ectodysplasin (Eda), a signaling molecule belonging to the tumor necrosis factor family, is required for normal development of several ectodermally derived organs in humans and mice. Studies with mice either lacking the functional proteins of Edar pathway or overexpressing the ligand or receptor suggest that Eda-A1-Edar signaling has multiple roles in ectodermal organ development regulating their initiation, morphogenesis, and differentiation [32]. Over expression of Eda-A1 resulted in supernumerary teeth and mammary placodes, which develop into mature organs [33]. Moreover, Eda-A1 transgenic embryos are characterized by increased placodal size, and treatment of embryonic skin with recombinant Eda-A1 in vitro promotes placodal cell fate in a dose-dependent manner [34]. Forced expression of Eda-A1 or Edar results in a lack of enamel in incisors [33] while high levels of expression of the transgene in wild-type mice result in molar teeth with extra cusps, and in some cases supernumerary teeth, the opposite of the mutant phenotype. The level of activation of Edar thus determines cusp number and tooth number during tooth development [35]. High levels of expression of Eda can cause supernumerary teeth and *Shh* signaling plays a key role in the process of tooth formation. Ectodysplasin can induce the expression of *Shh*, which suggests that *Shh* is a likely transcriptional target of Edar [36].

The canonical Wnt signaling also regulates the number of teeth. In β -cat ^{Δ ex3K14/+} mice, supernumerary teeth were formed not only by branching of dental epithelium but also by multiple placode formations [37]. Because β -cat ^{Δ ex3K14/+} mice were created by cross-mating *K14-Cre*^{Tg/+} mice and β -cat ^{Δ ex3flox/+} mice, the entire epithelial tissue including oral ectoderm and dental epithelium was expressing stabilized β -catenin, which mimics the continuous activation of Wnt/ β -cat signalling. Therefore the multiple placode formation in epithelium of β -cat ^{Δ ex3K14/+} mice might be occurred in part through a common mechanism in other Wnt signalling arranged mouse models of ectodermal appendages.

Epfⁿ KO mice develop supernumerary teeth formation in both incisors and molars [8]. Especially, Epfn KO mice keep developing supernumerary incisors with aging and we found nearly 50 incisors in 6-month-old Epfn KO mice [8]. The dental epithelial cells deficient of Epfn fail to polarize and to gain rapid proliferation

activity. No enamel formation is observed in *Epfn* KO mice due to the disturbing the dental epithelial cell differentiation into enamel-secreting ameloblasts. Undifferentiated dental epithelial cells in mutant mice sustain the immature states of cell physiology such as those in bud stage of tooth development. Dental epithelium of mutant mice keeps invaginating into mesenchymal tissue randomly and the interactions between dental epithelium and mesenchyme were sequentially occurred [8]. The mesenchymal cells adjacent of undifferentiated dental epithelium were induced to differentiate into dental mesenchymal cells that further differentiating into odontoblasts. The randomly induced odontoblasts in mutant mice start producing dentin matrix and formed tooth structures such as dentin and dental pulp. This finding suggests that the dental mesenchyme can be induced by interaction with undifferentiated dental epithelial cells and there is no limitation of the dental mesenchymal tissue production.

5 Conclusions

A variety of molecules contribute to form a functional shape and size of tooth and regulate the proper number of tooth. We have uncovered novel diverse roles of *Epfn* in tooth development. *Epfn* is essential for tooth morphogenesis by regulating tooth numbers and dental epithelial cell fate. Our studies provide new dimensions of understanding of the molecular mechanism governing the complex processes in tooth morphogenesis. Furthermore, our findings lead to develop a novel tissue engineering technique of the creation of bioteeth.

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Preparation and Biomedical Application of Self-Organized Honeycomb-Patterned Polymer Films

Takahito Kawano, Nagayoshi Iwama, Hiroshi Ishihata,
Hidetoshi Shimauchi, and Masatsugu Shimomura

Abstract. We found that self-organized honeycomb-patterned porous polymer films (honeycomb films) prepared by casting polymer dissolved in a water-immiscible solvent under high humidity. We demonstrated that the microtopography of the honeycomb film strongly affected human mesenchymal stem cells (hMSCs) and periodontal ligament (PDL) cells, which were the important cell sources for tissue engineering. hMSCs on the honeycomb films having small-sized (1.5 μm) pores induced a dramatic stem cell spheroid formation. PDL cells on the honeycomb films (pore sizes of 10 μm) formed increasingly elongated cell shape to trap in their pores. The honeycomb films, which controlled cellular morphology by changing only the geometric cues without the inducing media, can be applied as functional biomaterials for the regenerative therapy.

Key words. Cellular morphology, Honeycomb-patterned porous polymer film, Mesenchymal stem cell, Periodontal ligament cell, Self-organization, Topology

T. Kawano

WPI-AIMR, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan

N. Iwama, H. Ishihata, and H. Shimauchi

Division of Periodontology and Endodontology, Tohoku University

Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

M. Shimomura (✉)

WPI-AIMR, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan
and

Institute of Multidisciplinary Research for Advanced Materials, Tohoku University,
2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan

e-mail: shimo@tagen.tohoku.ac.jp

1 Introduction

Tissue engineering has improved to replace, repair and maintain damaged tissue function due to disease [1]. Regeneration of tissue function focuses on the optimization of the three main tissue engineering components: cell source, cytokine and scaffold [2]. It is known that the scaffold materials play a role in not only providing mechanical support to the cells but regulating cellular behaviors, such as cellular adhesion, migration, proliferation, and differentiation [3]. The engineering of cell scaffold to control the cell behaviors is expected to be a key technology for the development of a functional platform for biomedical application as well as for the systematic investigation of the mechanism of cell-biomaterials interactions. For the development of the functional biomaterials, it is important to focus on the biomaterials surface properties, such as bimolecular density [4], rigidity [5] and topography [6]. From the perspective of development of functional biomaterials, the topography is expected to be applicable for preparing an artificial extracellular matrix to manipulate cell function, because the nano/micro-geometric patterns can be designed using engineering approach. Recently, cell-cultured substrates with geometric sub-cellular patterns have been fabricated by various fabrication methods, such as electron beam lithography, and have been extensively used to investigate how cells respond to surface properties [7]. However, these techniques require a large amount of energy and involve many processes.

We have found that honeycomb-patterned porous polymer films (honeycomb films) were prepared by self-organization [8]. The honeycomb films have hexagonal packed regular sized micro-pores like in “honeycomb” and geometric double-layered structures supported by pillars (Fig. 1). Fabrication of honeycomb film has great advantages that the films can be prepared with simple process, at a low cost, and with less limitation of materials for cell scaffolds. Here, we discussed the effect of topography of honeycomb films on stem cell and periodontal ligament cell for the development of the functional biomaterials for the tissue engineering based on the regenerative therapy.

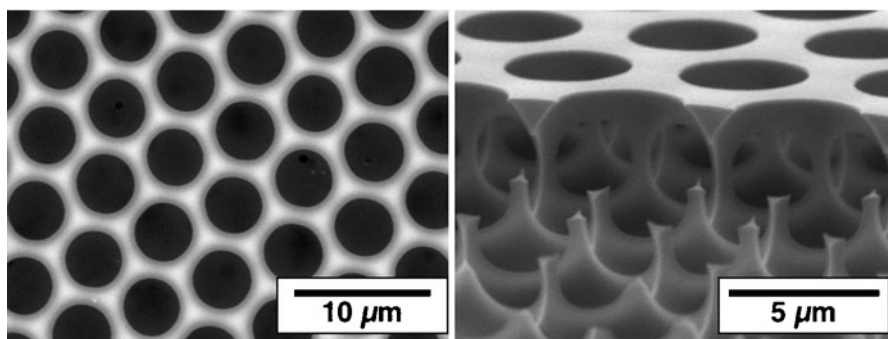


Fig. 1. Scanning electron microscopy (SEM) images of the top (*left*) and the cross-section (*right*) of the honeycomb film

2 Preparation of Honeycomb-Patterned Porous Film

The honeycomb films were prepared from water-immiscible solvent under humid casting condition of polymer solutions [9, 10] (Fig. 2). Condensation of water due to evaporation cooling was occurred during polymer casting. Self-packed surface monolayer of micro-dispersed water droplets formed on the solution surface act as a temporary template of micro-pores. Hexagonally packed regular sized micro-pores were formed in the polymer film after evaporation of solvent and water. The honeycomb film had the geometric double-layered structures supported by pillars. Pore size of the honeycomb films can be widely regulated from several decade microns to a few hundred nanometers by changing evaporation time or humidity and so on. Furthermore, the honeycomb films can be prepared from biodegradable polymers for the biomedical application [11].

3 Effect of Topography of Honeycomb Film on Stem Cell

We investigated how cells cultured on the honeycomb films would respond to changes in topography. Previously, human mesenchymal stem cells (hMSCs) have been shown to respond to surfaces with geometric patterns [12]. We prepared the honeycomb films of polystyrene with different pore sizes by changing the evaporation time, and hMSCs cultured on prepared honeycomb films for 2 weeks. The honeycomb films with pores size of 5 μm promoted adhesion, well-spread and formation of highly actin stress fibers (Fig. 3). In contrast, hMSCs on the honeycomb films

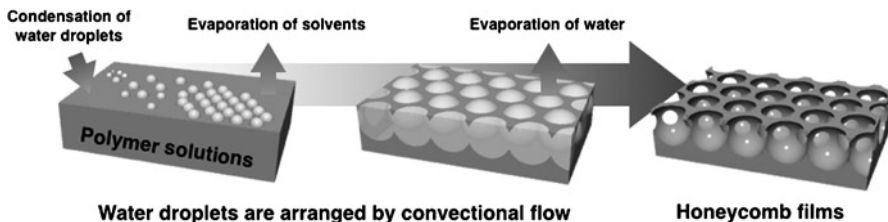


Fig. 2. Fabrication process of the honeycomb films

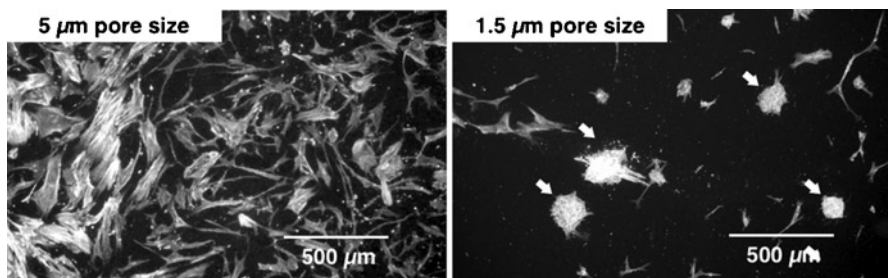


Fig. 3. Fluorescence images of the morphologies of hMSCs visualized fluorescence labeled actin fiber on honeycomb films after 2 weeks cultured. Arrows indicated the spheroid formations

with 1.5 μm pores enhanced the selective formation of stem cell spheroid (Fig. 3). We demonstrated that the stem cell spheroid formation controlled by changing of pore sizes of honeycomb films without the inducing media.

4 Application of Honeycomb Film for Periodontal Regenerative Therapy

Periodontal ligament (PDL) cell has the multipotency and self-renewal property like stem cell for the periodontal regenerate therapy [13, 14]. The honeycomb films combined with PDL cells were expected to provide as bioactive scaffold for periodontal regenerative therapy. To investigate the effect of honeycomb films on PDL cells, we observed of cell morphologies on the honeycomb films. PDL cells were isolated from the human teeth, and were cultured on the honeycomb films and flat films prepared by poly(ϵ -caprolactone). After 24 h, PDL cells on the honeycomb films having pores of 5 μm were spread on their surfaces and, had similar in morphology to cells on the flat films (Fig. 4). On the other hand, in the case of the honeycomb films having pores of 10 μm , PDL cells were moved and trapped in double-layered structures of honeycomb films and, formed increasingly elongated cell shapes (Fig. 4). These results suggested that morphologies of PDL cells could manipulate by changing pore size of honeycomb films.

5 Conclusions

Microtopography of the honeycomb film had a strong influence on the morphology and adhesion of hMSCs and PDL cells. The honeycomb film with an optimal pore size is expected to apply the functional biomaterials required for tissue engineering based on the regenerative therapy. Furthermore, since the honeycomb films easily are modulated the surface microtopography and the polymer materials, we can perform to systematically investigate of the mechanism the cell-topography of biomaterial interaction to manipulate cell behaviors using the honeycomb films.

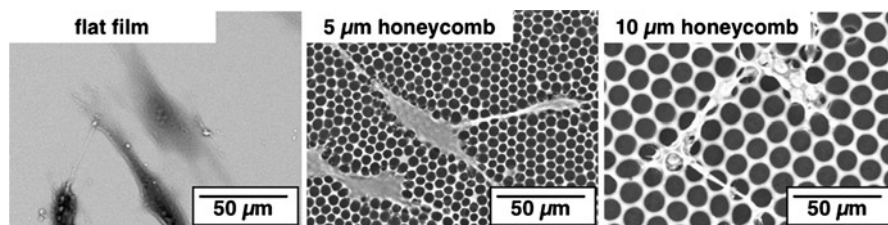


Fig. 4. SEM images of PDL cells on the flat film and the honeycomb films after 24 h cultured

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Symposium II
Current Activity in Research
and Education in Asian Graduate Schools

The Dental School We Aspire to Work for, Be Part of, and Invest Our Future in

Sunhun Kim

Abstract. The important message in dentistry is that our point of view needs to be modified and up-dated as dentistry has been changed with modern technology and complexity. Clinical dentistry is somewhat an art to apply technology, skills and knowledge about materials, diseases and tissues for dental patients. But, it also requires to embrace a better understanding of human behavior and social sciences. Chonnam National University School of Dentistry wants to be “The Dental School We Aspire to Work for, Be Part of, and Invest Our Future in”. Strategic plans for this are advanced education, cutting edge research, specialization and globalization. We highly recommend establishing an interface relationship between Chonnam National University School of Dentistry and other schools for better oral health science. Tohoku university has been the best example for the interface.

Key words. The Dental School We Aspire

The important message in dentistry is that our point of view needs to be modified and up-dated as dentistry has been changed with modern technology and complexity. Research is an essential key factor for all innovation in science. Research in dentistry includes even policy development and strategic planning for public dental services, besides basic investigation and clinical research. To research in dentistry is a process to find a relationship between interface structures. Furthermore, to care or treat dental patients is to improve a relationship between two parts or maintain a sound relationship.

Clinical dentistry is somewhat an art to apply technology, skills and knowledge about materials, diseases and tissues for dental patients. But, it also requires each of

S. Kim (✉)
School of Dentistry, Chonnam National University,
Yongbongdong, Gwangju 501-190, South Korea
e-mail: ksh@jnu.ac.kr

us to embrace a better understanding of human behavior and social sciences. Dentistry includes interface social science such as communication skills, empathy and cooperation between doctors and patients. Liberal arts is a necessity in dental education, as all activities in dentistry are for humanities.

Chonnam National University School of Dentistry wants to be “The Dental School We Aspire to Work for, Be Part of, and Invest Our Future in”. Strategic plans for this are advanced education, cutting edge research, specialization and globalization. Recently, the education system was changed from 6 year undergraduate to 8 year graduate system (2005), the new dental school and hospital building where handicapped and internal medicine patients can be also cared have been established (2008, 2011). Funding is an indicator of success in research. Brain Korea (BK) 21 national project has been undertaken for multidisciplinary research and education for 6 years. Doctor of Dental Surgery (DDS)-PhD program, a national training program for human resources for dental research has been run for 5 years. Medical Research Center (MRC) which is the biggest project of 7 years in dental fields has recently launched for the high quality of research. We highly recommend establishing an interface relationship between Chonnam National University School of Dentistry and other schools for better oral health science. Tohoku university has been the best example for the interface.

Introducing the Metabolomics Method into Oral Science to Find Something New

Jinglin Zhou and Wei Li

Abstract. Metabolomics and metabonomics, as one of omics techniques, have been widely applied in many system bioscience researches for recent decades, such as disease diagnosis, toxicology, pharmaceutical and environmental research. Combining the advanced detection methods (either Nuclear Magnetic Resonance spectroscopy or Mass spectrometry) and multivariate pattern recognition techniques, it can be detect the characteristic metabolomic profile from biofluids or tissue. The metabolites as a “signature” can display the result from the role of genes and proteins, and also can be informed the dynamic “signals” from disease or abnormal condition of organism. Oral cancer, periodontal disease and dental caries disease are still influence the oral health in the world. According to a WHO publication [21], oral health is one of focus on priority to solved problems. This publication suggests the aim of our researcher need to action. The metabolomics and metabonomics method can provide us an integrated view of biochemistry during the process of oral disease developing. It would give us a chance to make earlier diagnosis for oral cancer, stop the development during the premalignant lesions. It would help us find the factors lead the gingivitis reverse to periodontitis. It would find effect method to keep oral health easier.

Key words. Metabonomics, Metabolomics, Oral Science, Oral disease

Metabolomics and metabonomics, as one of omics techniques, have been widely applied in many system bioscience researches for recent decades, such as disease diagnosis, toxicology, pharmaceutical and environmental research. Compare with the traditional “bottom–top” method, which Genomics and Proteomics are proved,

J. Zhou and W. Li (✉)
State Key Laboratory of Oral Diseases, School of Dentistry, Sichuan University,
Chengdu, 610041, China
e-mail: leewei@scu.edu.cn

Metabolomics provides a “top–down” integrated view of biochemistry in complex organisms [1]. Metabolite profiling not only produces information on the biochemical pathways affected but also can produce time course information. With the rapid development of bioinformatics and cheminformatics, assessment metabolic response of biological systems and identify the structure of the character metabolites would be developed an effective clinical technique for human disease.

1 Methodology

Metabolomic profiling workflow as follow: firstly, make the study design and special requirement of subject. Secondly, collect and prepare samples using the protocol. The third step is detection of samples and collection of spectra data. The last one is analysis and figure out the important bioinformation from the matrix data.

1.1 Samples

Metabolomics can be estimate the disease from both of in vitro and in vivo using cells, fluids, or tissues. As the easiest sample preparation, biofluids are the most popular to be used, such as plasma, urine, saliva, ascitic fluid, cerebrospinal fluid, fecal water and so on. Because of the susceptibility of metabolomics profiling, the protocol of preparation of sample play an importance role in metabolomics methods. How to maintaining low temperature, what kind of special requirements for subject, how to keep standard process of sample preparation, such kinds of methods for collect and handling samples have been informed by most literatures [2–6].

1.2 Detection

Usually, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are main technologies to detect samples. NMR always be used to detect the hydrogen atoms in metabolites, and the biological fluid doesn't require any physical or chemical treatment prior to the analysis. It is a nondestructive method. While MS require the metabolites to be separated from the biological fluid before detection, typically, conjunct with gas or liquid chromatography. It is more sensitive than NMR. Obviously, each technique has each advantage and shortage.

1.3 Analysis

Matrix data from metabolomic profiling has a complex biological interconnection and reaction of each other. It not only need to simultaneous measurement but also need use chemometrics methods to analyze. Many chemometrics approach have

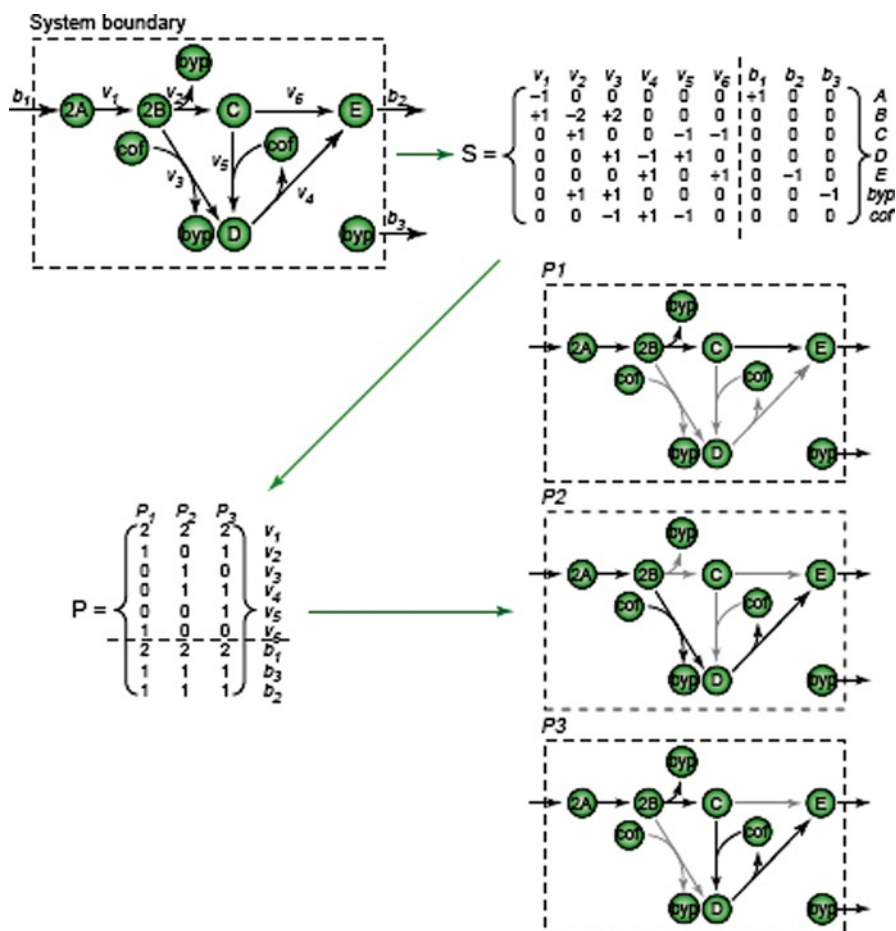


Fig. 1. Diagram of matrix with reaction chemometrics

been proved [7–14]. There are two major part of chemometrics approach be used, one is termed “Multivariate regression methods”, which are usually be popular used, including principal component analysis (PCA), nonlinear mapping (NLM) and hierarchical cluster analysis (HCA), partial least squares (PLS) and orthogonal projections to latent squares (OPLS), the other is termed “Machine learning techniques and non-linear methods” including neural networks (NN) and support vector machines (SVM). Figures 1 and 2 demonstrated the principle of chemometrics method: Figure 1 show a matrix with reaction chemometrics as columns and metabolite participations as rows, the methods of extreme pathway and elementary mode analyses can be used to generate a unique set of pathways P_1 , P_2 , and P_3 [15].

Figure 2 explain the steady-state operation of the metabolic network is restricted to the region within a cone, defined as the feasible set. The feasible set contains all

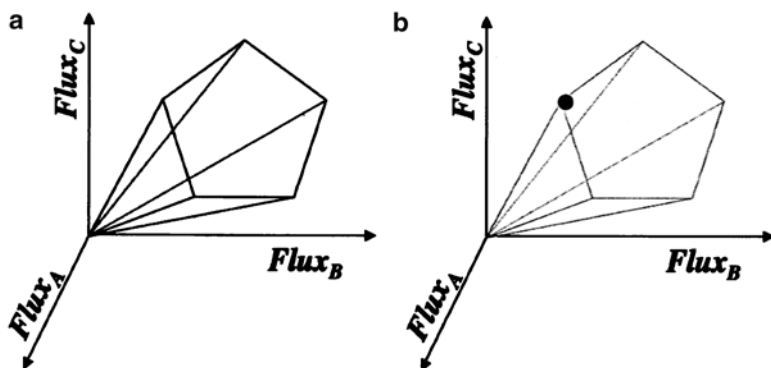


Fig. 2. Steady-state operation of the metabolic network

flux vectors that satisfy the physicochemical constraints. Thus, the feasible set defines the capabilities of the metabolic network. All feasible metabolic flux distributions lie within the feasible set in the limiting case, where all constraints on the metabolic network are known, such as the enzyme kinetics and gene regulation, the feasible set may be reduced to a single point. This single point must lie within the feasible set [16].

1.4 Metabolomic Database

NMR- and MS-based metabolomic experiments generate the complex data that contain thousands of signals, which represent a snapshot of the metabolite profile for a characteristic biological state. As a computational tool, easy to explain chemical structures, spectral data, and pathway diagrams. Extensively linked to other databases can provide the best comprehensive information for each tested metabolite [17]. A good database must be easily accessed online through a web interface, standardized data formats in describing, user-friendly interface to retrieval and visualization, and convenient exchange to other database.

2 Metabolomics as a New Method in Oral Disease

Oral health, as a special session, was organized by the WHO GOHP in the 7th WHO Global Conference on Health Promotion (2009, Kenya). Aim to oral health promotion and disease prevention, Oral health is a human right and essential to general health and quality of life [18]. Oral squamous cell carcinoma (OSCC) is the sixth most common type of carcinoma in the world. It is one of the malignant tumors accounting to 5.5%, and one of the most disfiguring types of cancer. Oral leukoplakia

(OLK) is characterized by the white premalignant lesions on the oral mucosa, it often has an obvious tendency of malignant transformation to OSCC. The relationship of the metabolites between OLK and OSCC must be identified so that the malignant transformation can be prevented by a prompt initiation of an adequate treatment. The ^1H NMR spectra were obtained from plasma of OSCC patients, OLK patients and healthy. The ^1H NMR spectrum of plasma revealed great complexity and significant information of the biofluid. The result [19] of the PLS-DA analysis has revealed a good model to detect the NMR data that can differentiate the OSCC patients from the OLK patients and the controls by using a test set.

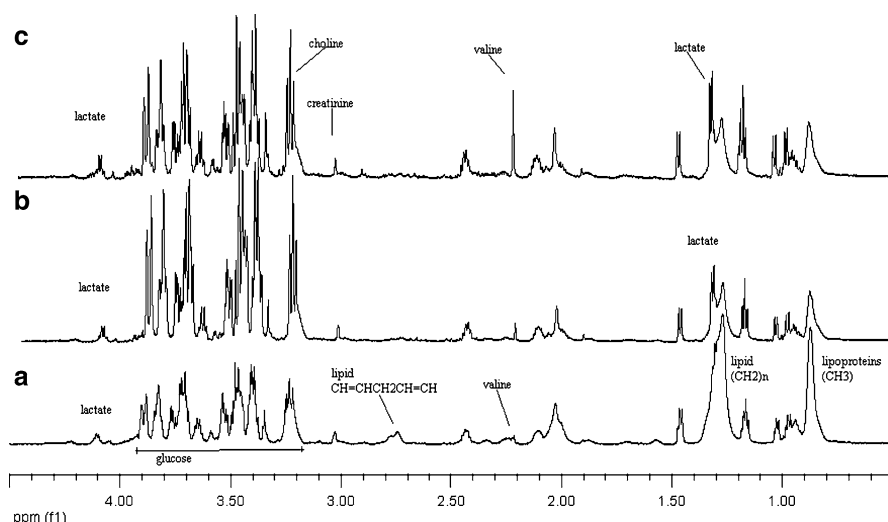


Fig. 3. ^1H NMR spectra of plasma of OSCC patients, OLK patients and healthy

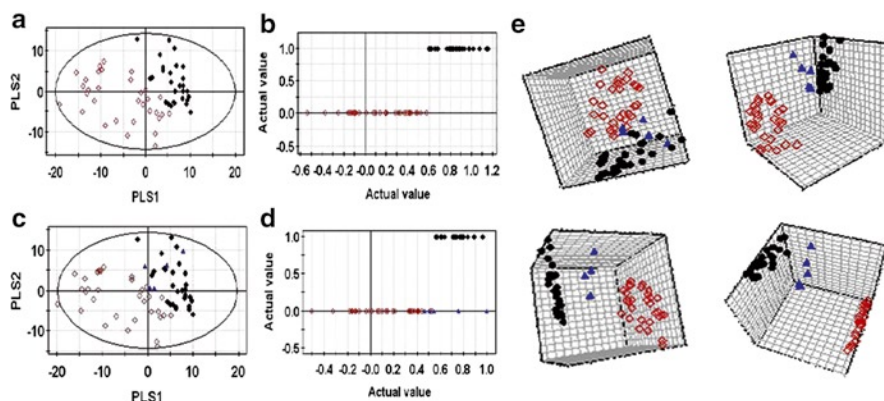


Fig. 4. PLS-DA analysis of OSCC patients, OLK patients and healthy

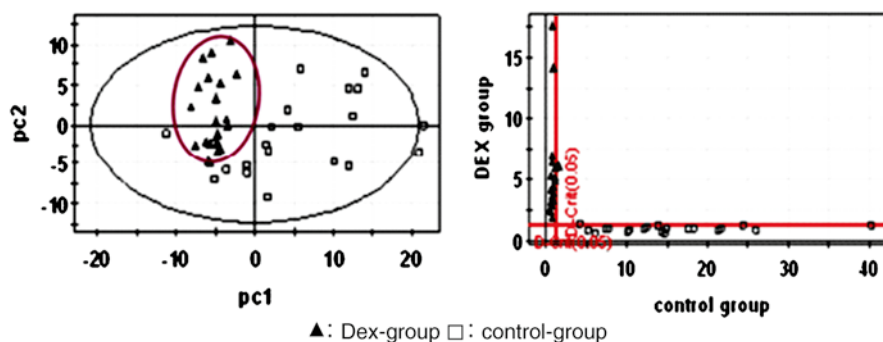


Fig. 5. PCA and SIMCA analysis of DEX-induced CLP mouse model. *Filled triangle* Dex-group, *open square* control-group

Congenital cleft lip and palate (CLP) is the most common birth defect in humans. Maternal condition during pregnancy also appears to play an important role. Dexamethasone (DEX) can penetrate the blood-placental barrier and bind to GC receptor (GR) in the cytoplasm, and cause depress the ability of palatal mesenchymal proliferation, mouse model are used to assess the mechanism of palate defects in fetuses resulting from exposure to the risk factors. Metabonomics, based on the NMR spectroscopic and multivariate statistics, can be useful for the description and recognition of the dynamic multivariate metabolic response of an organism to a pathological event or genetic modification [20].

Other oral disease, such as dental caries, gingivitis and periodontitis, are characterized related with the balance of oral microbiome system. Oral microbial-plaque communities are biofilms composed of numerous genetically distinct types of bacteria that live in close juxtaposition on host surfaces. Estimate the diversity of metabolites can inform the characteristic change of oral microenvironment. It can be easy to understand oral microbial pathogenic mechanism.

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Eight-Year Program of Stomatology Education in China

Guo Chuanbin, Liu Hongwei, Jiang Yong, and Xu Tao

Abstract. The 8 year stomatology education is a new educational system in China, which is divided into two stages: the first 5-years for preclinical, clinical medicine and basic dentistry training and the second 3-years for resident dentist training. Students are required to complete a clinical based research study in the last year. Ability oriented education is emphasized. Doctoral Degree of Medical Science (Stomatology) is granted for students who finish all courses and meet all requirements. Two classes of students already graduated from our school. Their performances met the original purposes of this program which is to not only train a future dentist but also help them to know the fundamentals of research skill as well as social and culture knowledge to prepare them to become an important addition to the society.

Key words. Stomatology, Dentistry, Education

1 Introduction

The primary medical education program in China is a 5 year program. An 8 year program of medical education is a new educational system introduced in a few top universities in China. We began this program in 2001 and graduated two classes of students already. Since then, some other stomatology schools in China began to follow up the same program.

The main contents of this article include: targets of this new stomatological education, allocation of 8 years, main courses, key education points, requirements for graduation and grant of academic degree, and related reforms for the program.

G. Chuanbin, L. Hongwei, J. Yong, and X. Tao (✉)
Peking University School of Stomatology, Zhongguancun South Ave. 22,
Beijing 100081, China
e-mail: guodazuo@sina.com

2 Construction of the 8 Year Stomatology Education System

2.1 Basic Requirements

1. *Professional attitudes and moral character*: Students should have scientific views, devotion to the profession, excellent medical ethics, interesting in science and obey the law and regulation.
2. *Knowledge requirement*: Students should have knowledge of both cultural sciences and medical sciences.
3. *Capability*: Students should have clinical ability of senior resident dentist, research ability of clinical or basic scientific research, sense of innovation and certain ability of international communication.

2.2 Allocation of 8 Year

Eight-years curriculum is divided into two stages: the first stage for 5 years and the second year for 3 years.

1. *The first 5 years*: Common courses 1 year (mathematics, chemistry, physics, psychology, etc.), basic biomedicine courses 1.5 years, clinical medicine courses and clinical practice 0.75 year, stomatology courses and practice 1.75 years
2. *The second stage 3 years*: Resident dentist training 3 years.

2.3 Main Courses

Platform: English, philosophy, computer, physical training, etc.

Occupation and cultural sciences: Psychology, law, ethics, doctor–patient relationship, early clinical experience, etc.

Natural sciences: Chemistry, physics, higher mathematics, general biology, etc.

Public health: Prophylactic medicine, epidemiology

Scientific research related: Biostatistics, medical statistics, application of SPSS, literature search and review, evidence based medicine, clinical epidemiology, clinical research methodology, etc.

Preclinical medicine: Human anatomy, histology, embryology, physiology, biochemistry, cell biology, immunology, genetics, pathophysiology, pathoanatomy, pharmacology, biomedicine experiment, etc.

Clinical medicine: Diagnostics, internal medicine, surgery, gynecology, pediatrics, ophthalmology, ENT, dermatology, venerology, Chinese medicine, etc.

Stomatology (Dentistry):

- (a) Dental anatomy, oral physiology, oral histopathology, dental material science, oral radiology, oral biology, oral clinical pharmacology, dental equipment
- (b) Odontology and endodontics, periodontics, pediatontology, diseases of oral mucosa, oral and maxillofacial surgery, prosthodontics, orthodontics, preventive dentistry, oral implantology

2.4 Main Teaching Points

The main purpose of this teaching system is to raise students' ability of self-renewal for new knowledge. The following teaching points are focused.

Common courses, English, preventive medicine and evidence-based medicine teaching are strengthened. For preclinical and clinical courses, students are required to have early clinical touch. For experiment courses, students are advocated to have independent design of various experiments. For clinical training, students are given more time to practice for raising clinical abilities. Modern teaching techniques like PBL, CBL, multimedia and web, are applied. Different ways of examination are used to evaluate students' performance.

After finishing the first stage training, students enter the second stage. During the second stage, students attend all academic activities of the school, with focus on clinical training. Students are required to finish a clinical based study in the last year.

2.5 Thesis

Minimal require: A case report with literature review. Clinical study is preferred.

2.6 Graduation Requirements and Grant of Academic Degree

Relative elimination: If students do not meet requirement of credit points, fail to complete school work in 8 years, fail to pass English exam, fail to pass stage one or two exam, and have punishments because of demerits, they may be eliminated from the program.

Both Bachelor and Doctor degree of Medical Science are granted when students complete all curriculums, pass all exams, have no punishments, succeed in thesis defense. If students only finish the first stage study and do not continue the second stage with success in all required exams of the first stage, only Bachelor degree of Medical science is granted.

3 Eight Year Program Related Teaching Reforms

The teaching reforms include curriculum reform, teaching materials improvement, teaching methods reform, early touch of scientific research, ability oriented education, etc.

3.1 Curriculum Reform

Traditional stomatology education in China does not have dentistry education in the first 3 years. To raise students' interest in dentistry, we allow students have early contact in general dentistry, early rotation in dental clinical, basic statistic study, etc. in the first and second school years.

3.2 Teaching Methods Reform

Ninety-eight percent teaching use multimedia technique, 90% courses have multimedia courseware available on web, 33% use PBL, and 30% have bilingual teaching.

3.3 Teaching Researches

For this new education system, we have 3 national level teaching research projects, 6 university level teaching research projects, 85 school level teaching research projects.

3.4 Early Contact for Scientific Research

Two opportunities for students to have early contact and gain early experience for scientific research: during preclinical medicine and before enter dentistry study. Each student is funded with a seed grant for their research project.

3.5 Ability Oriented Education

One's ability is a basic requirement for good performance in this world. Methods of ability oriented education include professional ethics education, learning more knowledge of the humanities, enhancing the abilities of practice, communication,

self-learning, and innovation, creating a good school atmosphere, establishing a high quality group of teachers.

Students have 1 year study of the humanities knowledge in Peking University and should learn this kind of knowledge through 8 year study. Elicitation teaching methods replace the spoon-feeding way of teaching; PBL is one of the methods, which is used for exploitation of initiative and creativeness. Students are encouraged to have early contact with patients, participate in community clinical service to build clinical practice ability, to attend to dental public health activities in the community, to visit other schools and participate or organize meetings or conferences for developing communication and organizing ability.

A high quality group of teachers is also very important. In our school teachers should be experienced with excellent teaching record. They are required to not only have regular training skill for teaching students but also asked to pass a quality evaluation.

4 Summary

The 8 year stomatology education is a new educational system in China, which is divided into two stages: the first 5-years for preclinical, clinical medicine and basic dentistry training and the second 3-years for resident dentist training. Students are required to complete a clinical based research study in the last year. Ability oriented education is emphasized. Doctoral Degree of Medical Science (Stomatology) is granted for students who finish all courses and meet all requirements. From the learning from the first two classes of graduates from our school, we think this program is in general meet the original design which is to not only train a future dentist but also help them to know the fundamentals of research skill as well as social and culture knowledge to prepare them to become an important addition to the society.

Interdisciplinary and International Research/Education Based on Interface Oral Health Science

Nobuhiro Takahashi

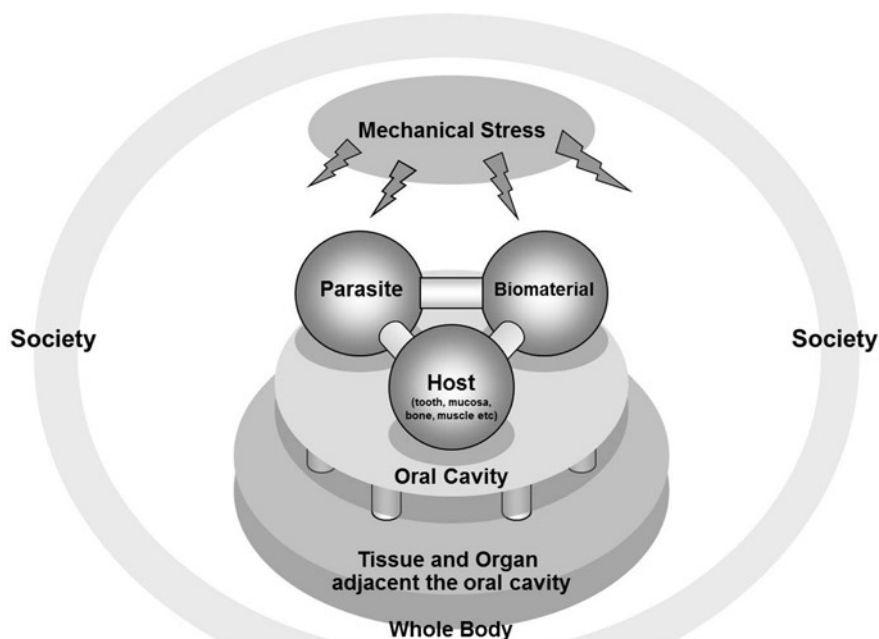
Abstract. Based on the new concept of “Interface Oral Health Science”, Tohoku University Graduate School of Dentistry has performed (1) interdisciplinary research/education, such as the integration of biological dentistry and material science, and (2) international research/education through international symposia/conferences where graduate students participate as active researchers. In addition, two international programs of graduate education, the Global 30 Program (year 2011) and the Double-Degree Program (year 2012) are expected to launch for overseas students. Interdisciplinary and international research/education is essential for oral science in the future, since oral science is closely related with other research fields and can be developed through mutual and synergistic interactions with other research fields.

Key words. Interdisciplinary, International, The Double-Degree program, The Global 30 program

In 2002, Tohoku University Graduate School of Dentistry established “Interface Oral Health Science”. This is a new concept in which healthy oral function is provided by biological and biomechanical harmony among three systems: oral tissues, parasites, and biomaterials, and oral diseases are interpreted as “interface diseases” caused by disruption of the intact interfaces among those systems. Oral health will be finally achieved in our society when individual people can be provided with therapy, prevention and education, so “social interface” is an additional interface to be considered (Fig. 1). Based on this concept, our graduate school has performed (1) interdisciplinary research/education such as the integration of biological dentistry

N. Takahashi (✉)

Division of Oral Ecology and Biochemistry, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: nobu-t@dent.tohoku.ac.jp



**Healthy oral function is achieved by the biological and biomechanical harmonization of interfaces between host tissue, parasite and biomaterial.
Oral health is promoted by interaction with society.**

Fig. 1. “Interface Oral Health Science” as a concept of future dental research, since 2002 in Tohoku University Graduate School of Dentistry. Modified from [1]

and material science, and (2) international research/education through international symposia/conferences where graduate students participate as active researchers. In addition, two international programs of graduate education, the Global 30 (G30) Program (2011) and the Double-Degree (DD) Program (2012) are expected to launch for overseas students.

The Japanese government (MEXT) launched the G30 Program in 2009, to create a high-quality international educational environment. Tohoku University is among the universities selected for the G30 Program, in which, Ph.D. students study for 4 years in English to earn a Ph.D. from Tohoku University. The course will offer the “Interface Oral Health Science Course”, in which students attend lectures, and undergo research training and thesis writing in English at Tohoku University.

In addition, Tohoku University Graduate School of Dentistry will launch the DD Program on its own accord, in which, based on an agreement between Tohoku University Graduate School of Dentistry and a partner university, Ph.D. students will study at both universities in parallel and can earn two doctoral degrees from Tohoku University and the partner university. The DD Program aims to develop the next generation of researchers and clinical professionals in oral health sciences with international research and interdisciplinary knowledge as an East-Asia standard.

Interdisciplinary and international research/education is essential for oral science in the future, since oral science is closely related with other research fields and can be developed through mutual and synergistic interactions. In this environment, young oral scientists, as well as new oral science, can be developed.

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Dental Education in Mongolia

Amarsaikhan Bazar and Bayarchimeg Batbayar

Abstract. In 1961, Mongolian National Medical Institute set up the first dental education course enrolling students who were mostly nurses and train them for 3 years to become dentists. During that time the Department of Dentistry had been subordinate to other departments and on January 1, 2000 the School of Dentistry was established in Health Sciences University of Mongolia. Nowadays the School of Dentistry is preparing dental professionals at the national level. It consists of five academic departments: Currently there are more than 40 faculty teachers and 628 students enrolled in the program. The school has affiliated the Dental Hospital, which consists of: The Implant and Cosmetic dentistry Center, Dental Imaging section, Postgraduate Dental Clinic, Prosthodontic clinic, Operative clinic, Oral and Maxillo Facial surgery clinic. From 2007 it was established the Dental Research Institute. Research conducted at the School of Dentistry involves fundamental and clinical research which addresses problems and promotes oral health for the people of Mongolia. The school offers a 5-year undergraduate curriculum including from general (medical) education to basic dental and clinical education. Fifth-year dental students spend 80% of their time in direct, in-clinic patient care and 20% of their time in didactic study.

Dental services are inadequate everywhere to cope with needs, and separate organized services generally are not available. The dentists in private practice are concentrated in the urban centers. In Mongolia 79% of the country's 900 dentists live and work in Ulaanbaatar. Thirty percent of dentists are working in government service, and seventy percent in private practice. There are 160 dental technicians. It is necessary to train either a dental hygienist or a dental nurse.

A. Bazar (✉)

Department of Prosthodontics and Orthodontics, School of Dentistry, Health Sciences University of Mongolia, Choidog Street 3, PO Box 56, Ulaanbatar 210648, Mongolia
e-mail: bazaramarsaikhan@yahoo.com

B. Batbayar

Department of Restorative Sciences, School of Dentistry, Health Sciences University of Mongolia, Choidog Street 3, PO Box 56, Ulaanbatar 210648, Mongolia

During the historical 50-years of period the field of Mongolian Dentistry not only established its fundament but also achieved the recognition among colleagues from other field of sciences. Today, the School of Dentistry is well-known among international colleagues and has prepared more than 1,500 professional dentists.

Key words. Dental education in Mongolia, School of Dentistry

1 History of Dentistry and Dental Education

The origin of the field of Mongolian Dentistry can be tracked back to the first five professionals who graduated in former Soviet Union in 1955 [1]. In 1961, Health Sciences University formerly called Mongolian National Medical Institute set up the first dental education course enrolling students who were mostly nurses and trained them for 3 years to become dentists. Since the beginning of the course, the Institute has been preparing dental professionals at the national level.

During that time the Department of Dentistry had been subordinate to following departments: Department of Otorhinolaryngology (1961), Department of Pediatric Medicine (1962–1964), Department of Research Surgery (1964–1967). From 1967 the Department of Dentistry and Otorhinolaryngology started a 5 year training course for doctors of dentistry. From February 1, 1978 the Department of Dentistry became an independent department. On November 26, 1995 the Department of Dentistry challenged its name to “Center of Dental Research”. On January 1, 2000 the School of Dentistry was established in Health Sciences University of Mongolia (HSUM). The days School of Dentistry is well-known among international colleagues and has prepared more than 1,500 professional dentists. On September 1, 2006 Dental Technician course from Nursing School annexed to the School of Dentistry. In 2010 the Department of Dental hygienists was established in the School of Dentistry.

Nowadays the School of Dentistry, HSUM is governmental school and an integral part of the Health Sciences University of Mongolia. Beside of it on 2004 there was established private medical college with the Department of Dentistry in Ulaanbaatar.

The School of Dentistry is preparing dental professionals at the national level. It consists of five academic departments: Operative Dentistry, Maxillofacial Surgery, Prosthodontics and Orthodontics, Pediatric and Preventive Dentistry and Technology (Fig. 1). Currently there are more than 40 faculty teachers and 628 students, whereas 450 are dental students, 150 dental technician students and 28 dental hygienist students enrolled in the program. In addition to the Doctor of Dental Surgery (D.D.S.) degree, the School offers a 3 years PhD and 2 years Master degree programmes in Dentistry. The school has affiliated the Dental hospital, which consists of: The Implant and Cosmetic dentistry Center, Dental Imaging section, Postgraduate Dental Clinic, Prosthodontic clinic, Operative clinic, Oral surgery clinic. Residency training programs are available in Restorative

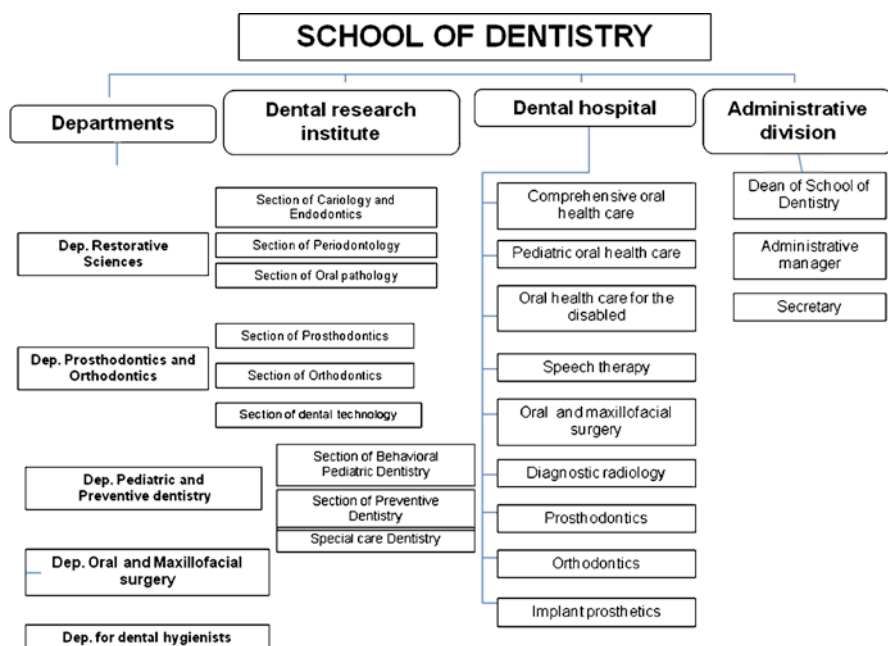


Fig. 1. The structure of the School of Dentistry, HSUM

Sciences, Pediatric and Preventive Dentistry, Prosthodontics and Orthodontics, Oral and Maxillofacial surgery. From 2007 it was established the Dental Research Institute. Research conducted at the School of Dentistry involves fundamental and clinical research which addresses problems and promotes oral health for the people of Mongolia.

2 Dental Enrollment and Curriculum

The Ministry of Education, Culture and Science supervises dental school. Applicants to School of Dentistry of HSUM can take statewide entrance examinations. In 2010, first-year dental students in dental school numbered 102 [2]. Eligible applicants must have graduated from high school and no undergraduate pre-dental study is needed prior to entry into dental school. All candidates applying to School of Dentistry must have examination in the areas of chemistry, and biology as well as English or Russian languages. The 2 days nationwide entrance exam consists of multiple choice questions, short answers. School of dentistry must make rapid admission decision based solely on the academic score achieved by applicants. The admission process is extremely competitive. More than 1,000 applicants compete for entrance to School of Dentistry, HSUM each year, but only a very small number

(102 in Year 2010) gain admittance. The entrance examination is provided once annually and is held only a few months before the programme starts. Applicants are required to undergo a medical examination to satisfy health requirements. In the recent past the number of admissions has increased. The number of entering dental students may increase further in the coming years. However, there are limited facilities to increase the enrollment number. There are 4.5–10 times as many applicants as there are student places available.

In Mongolia, the average first-year tuition and fees are 1,200,000 Mongolian tugric (US\$ 926) for School of Dentistry, HSUM.

Dental personnel in Mongolia comprise dentists, dental nurses, and dental technicians. The School of Dentistry, HSUM offers a 5-year undergraduate curriculum for dentists with 178 credits, 3 years for dental technician students with 105 credits and 4 years for dental hygienist students with 140 credits, including from general (medical) education to basic dental and clinical education. Dental students studying basic sciences during the first and second years of the curriculum and basic medicine during the third year. From fourth year curriculum students begin making contact with patients. Teaching and learning methods are lectures, seminars and practical sessions. Teaching in the basic science subjects is still done by other school departments of HSUM. During their clinical training at the affiliated hospital, students are provided with detailed and effective education as they rotate through the different departments. This enhances their appreciation and knowledge of the human being and medical services. Due to the socioeconomic conditions in Mongolia implant, laser dentistry, precious metal casting and etc. taught in post-graduate course. School of Dentistry requires a final qualifying exam at the end of the each subject course of study. The theoretical knowledge is evaluated by final written or verbal exams. The maximum score for an examination is 100, and a minimum passing score of 60% is necessary. Fifth-year dental students spend 80% of their time in direct, in-clinic patient care and 20% of their time in didactic study. In the past students used Russian dental textbooks written in Russian language. From 2005 dental school staff began to produce Mongolian language dental textbooks. National Board of Examination consists of written and practical manual examination including both theory and clinical elements. Assessment of the practical skills and competences performed using eight steps theoretical and practical–phantom skill examinations and written tests. Dentist graduates are awarded as a doctor of dental surgery degree (D.D.S.). Dental technician graduates are awarded as a bachelor of dental technician degree (B.T.S.). And dental hygienist graduates will be awarded as a bachelor of dental hygienist degree (B.D.D.H.). Two years master and three years PhD programmes are also available. The total number of freshman dental students is about 120 per year. About 30 new dental technicians are graduated annually. Postgraduate dental education activities comprise vocational training, continuing professional education, and specialization in one of the clinical disciplines. School organizes continuing education short courses for 1–3 months every year.

3 Dental License and Practice

Dental school graduates who wish to practice must be approved by the state board of dentistry or examiners for licensure. The Mongolian nationwide dental licensure examination conducted by the Licence division of the Government Implementing Agency–Department of Health and appointed expert prepare the written examination. The exam results are reported on the pass basis and a minimum score of 70% is required to pass.

The dentist-to-population ratio has improved over the years. School of dentistry has prepared more than 1,500 professional dentists in the period of 1965–2008 [3]. However, at present, there are about 900 dentists and 160 dental technicians are practicing, for a population of 2.7 million. During the next 50 years the population of Mongolia is expected to double, reaching nearly 5 million, and 2,500 dentists will be required for a ratio of 1 to each 2,000 persons. Allowing for those leaving the profession, a net annual output of 500 dentists per year is necessary. Dental services are inadequate everywhere to cope with needs, and separate organized services generally are not available. However the geographic distribution is of dentists is very uneven, being many more in the cities than in small rural area. The dentists in private practice are concentrated in the urban centers. In Mongolia 79% of the country's 900 dentists live and work in Ulaanbaatar. 30% of dentists are working in government service, and 70% in private practice. There are 160 dental technicians. It is necessary to train either a dental hygienist and/or a dental nurse. Last years dental care used nurses as dental auxiliaries and it was zero the output of dental hygienists for many years to come. Training dental hygienists directly from secondary school graduates has proved practical in countries that have an adequate supply of these graduates. Such dental auxiliaries can be used in two ways-as chair-side assistants and as semi-independent chair side operators. As semi-independent operators, they will be giving direct clinical care, under supervision which may be either close or remote, depending on the availability of professional dentists and the geographic isolation of the clinic.

During the historical 50-years of period the field of Mongolian Dentistry not only established its fundament but also achieved the recognition among colleagues from other field of sciences. Today, the School of Dentistry is well-known among international colleagues and has prepared more than 1,500 professional dentists.

4 Conclusion

- Mongolia is moving an economical development.
- The cost of imported dental materials and equipments still limits dental education and services.
- It is necessary to train either a dental hygienist or a dental nurse. Training dental hygienists directly from secondary school graduates has proved practical in

countries that have an adequate supply of these graduates. Such dental auxiliaries can be used in two ways-as chairside assistants and as semi-independent chairside operators. As semi-independent operators, they will be giving direct clinical care, under supervision which may be either close or remote, depending on the availability of professional dentists and the geographic isolation of the clinic.

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Symposium III
Highly Functional Biomaterials

Design of Biomaterials for Bone Replacement Based on Parameters Determining Bone Quality

Takayoshi Nakano and Takuya Ishimoto

Abstract. In addition to the bone mineral density (BMD), new parameters determining the bone quality have been investigated recently because bone is a well-organized hierarchical composite at various scale levels. BMD is nothing but the density of biological apatite (BAP); however, the crystallographic orientation of BAP crystallites corresponds to the rotation of BAP crystallites, and these two parameters are independent. In other words, the degree of BAP orientation might be a parameter determining bone quality. Thus, our group has studied the preferential degree of the BAP *c*-axis orientation in normal, pathological, and regenerated bones using microbeam X-ray diffraction.

The preferential degree of the BAP *c*-axis orientation strongly depends on factors such as the bone position, in vivo stress distribution, bone growth, degree of pathology and bone regeneration, turnover rate, activity of bone cells, and gene defects. Correlations are clearly observed among the BAP orientation, in vivo stress distribution, and mechanical function in normal, pathological, and regenerated bones, including mandibles.

Because of the anisotropy of bone microstructure based on the BAP orientation distribution, implants for bone replacement should be developed by taking into account the structural and/or material anisotropy. For example, an implant with unidirectional-elongated-through pores was implanted such that the elongated pore direction was parallel or perpendicular to the mesiodistal axis in the mandible with one-dimensional orientation of the BAP *c*-axis. The degree of calcification and the subsequent orientation of BAP during bone regeneration are greater in the elongated pore parallel to the mesiodistal axis than in the pore perpendicular to the axis, indicating that BMD and BAP orientation of the newly formed bone can be controlled by the elongated pore direction; this is closely related to the anisotropic bone microstructure and in vivo principal stress direction.

T. Nakano (✉) and T. Ishimoto
Division of Materials and Manufacturing Science, Graduate School of Engineering,
Osaka University, 2-1 Yamada-oka, Suita, Osaka 565-0871, Japan
e-mail: nakano@mat.eng.osaka-u.ac.jp

The electron beam melting (EBM) method, which is a rapid prototyping technique for developing anisotropic-shaped implants, is a promising candidate for artificially fabricating arbitrarily shaped nonporous and porous materials and even customized medical devices. This technique can be used to fabricate implants having arbitrary morphology from the raw powder metals without the use of a casting mould.

Because the BAp orientation distribution strongly influences the mechanical, chemical, and biological functions of bones, methods for controlling the BAp orientation should be developed.

Key words. Apatite orientation, Bone quality, Electron beam melting (EBM), Mandible, Microbeam X-ray diffraction

1 Introduction

With the increasing number of elderly people in society, the prevention and treatment of bone diseases and the establishment of methods to regenerate bone tissue are necessary for improving the quality of life of the elderly. For the replacement of defective bones, the use of metal implants is preferred from the viewpoint of mechanical reliability and safety. For the recovery of bone functions, on the other hand, the development of soluble materials with bone-regenerating capabilities is considered essential. Therefore, the development of materials combining both these characteristics is highly desired. At the same time, it is necessary to evaluate these materials from the viewpoint of their suitability for implantation and their capability for regenerative function in the surrounding bone tissue. However, while the nano-scale evaluation of bone is very important, studies on it have not been sufficient yet. In addition, it is difficult to design a biomaterial while taking into account the condition of surrounding bone tissues after implantation. Therefore, it is necessary to develop new biomaterials and establish bone evaluation techniques in a complementary manner; the goal of developing new biomaterials will remain unattainable if either effort fails.

Bone quality has attracted considerable attention with the increasing prevalence of osteoporosis. Bone quality is a concept that was proposed by the National Institutes of Health in 2000. It encompasses factors other than bone density, which indicates bone strength [1]. When this concept was introduced, the well-known parameters used for evaluating bone quality included the microstructural organization, bone turnover rate, occurrence of micro-cracks, and cellular properties. However, these criteria cannot be said to be necessarily sufficient for elucidating controlling factors of bone strength.

In this review paper, the alignment of biological apatite (BAp) is first discussed as an important parameter for evaluating bone quality. Subsequently, the development and design of biomaterials based on the structural anisotropy of bones is discussed, and an approach from the viewpoint of the anisotropy of bone tissues is introduced.

2 Orientation Degree of BAP as a Parameter for Evaluating Bone Quality

2.1 Microstructure of Bones and BAP Orientation Based on Stress Distribution

Bones are well-organized tissues that are mainly composed of an inorganic component, BAP, and an organic component, Type I collagen [2]. They also include skeletal cells that govern bone remodeling. The inorganic and organic components are responsible for the strength and pliability of bones, respectively. As shown in Fig. 1, BAP has a highly anisotropic structure with a hexagonal crystal system. Its c -axis aligns in a direction similar to that of fibrous collagen [3, 4]. As a result, the orientation of the BAP/collagen complex within bone tissues is considered a dominating factor that determines bone properties [4]. As shown in Fig. 2, cortical bones exhibit various geometries according to their regions, and they also exhibit different in vivo stress distributions. For example, the ulna, mandible, and vertebra of a rabbit and simian exhibit the BAP orientation alignment only in the longitudinal, mesiodistal, and craniocaudal direction, respectively. These tissues preferentially have a one-dimensional orientation along the BAP c -axis. In contrast, the skull bones of rabbits, which are flat, preferentially have a two-dimensional orientation of BAP along the bone surface. These particular alignments are strongly related to the in vivo stress distributions. In particular, the preferential direction of the BAP c -axis orientation corresponds to the principal stress direction inside the body, and changes

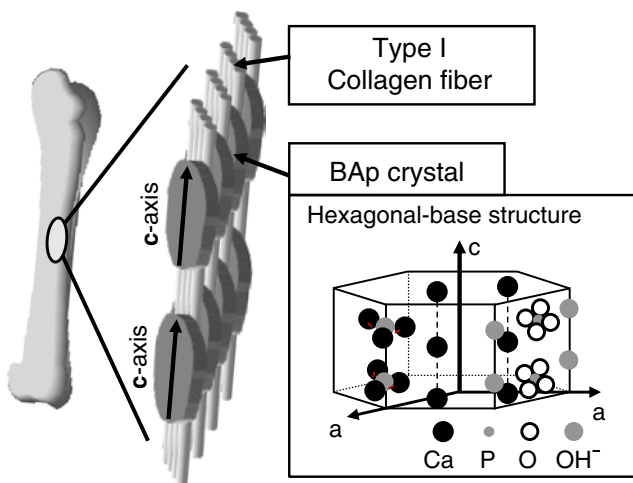


Fig. 1. Schematic illustration of bone dominantly composed of apatite crystal and Type I collagen at some scale levels. The c -axis of biological apatite (BAP) is aligned in a direction similar to that of collagen fiber [3, 4]. BAP crystallizes in the hexagonal-base structure with a high anisotropic ion arrangement

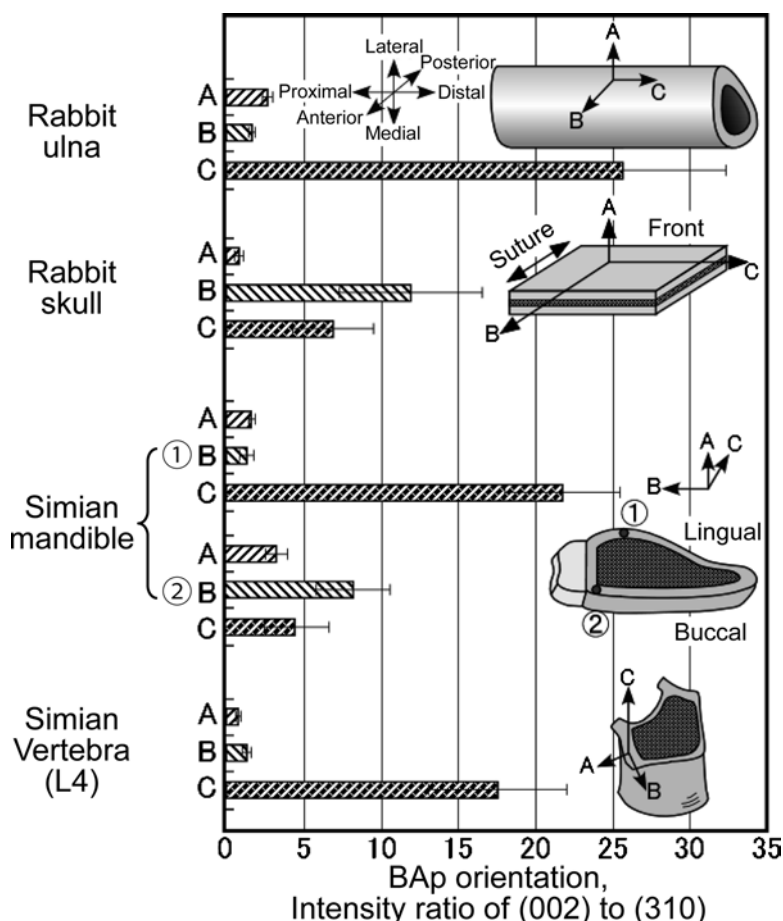


Fig. 2. Preferential degree of the BAp *c*-axis orientation in typical mature cortical bones. This figure was redrawn from [5]

even by the local *in vivo* stress distribution; for example, the difference between ① and ② of the mandible is closely related to the mastication stress [5].

Figure 3 shows the alignments of the BAp *c*-axis orientation directly under the tooth crown in a simian mandible. The alignments lie along A, B, and C directions [5]. The mandible basically has a preferential alignment of BAp orientation in the mesiodistal direction (C). However, immediately beneath the crown, the degree of this alignment rapidly decreases. Rather, in the mastication direction (B), the degree of BAp alignment shows the highest value because of the mastication load, also shown at position ② in Fig. 2. This trend is observed to be strong on the buccal side, which has a configuration that can easily withstand the mastication load. This proves that the change in stress distribution due to mastication directly controls the BAp alignment.

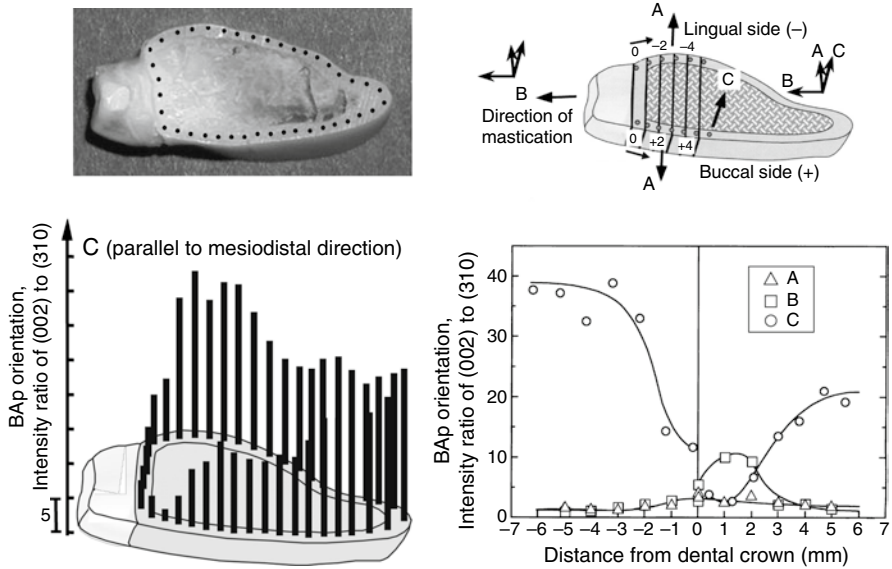


Fig. 3. Preferential degree of the BAp *c*-axis orientation in simian mandible with a tooth crown. The mandible basically shows a preferential alignment of BAp in the mesiodistal direction (C); however, under the crown, the direction of maximum preferential alignment of BAp changed to the direction of the mastication load (B). The relative intensity ratio of the (002) diffraction peak to the (310) one is around 2 in randomly oriented apatite. This figure was redrawn from [5]

In other words, the BAp/collagen complex of cortical bones changes the preferential alignment to support the *in vivo* stress distribution in various regions of the body, and it causes the bones to exhibit the most appropriate mechanical function depending on the region.

2.2 Change in BAp Orientation in Regenerated Bone

Remarkable progress has been made in the field of bone tissue engineering [6]. However, it remains unclear whether regenerated bone tissues produced in a forced and quick manner possess the same structural and functional properties as normal bone tissues. Thus, the analysis of the BAp alignment provides important information of the stress distribution and bone tissue properties. As a technique for evaluating the function of regenerated tissues, it is also useful for providing insights into their regenerative process [7].

For example, a complete 1-cm defect was introduced in the ulna of a rabbit. Several weeks later, the defect was perfectly covered over, and the regenerated portion looked as if it had recovered to its original condition [7, 8]. However, even while the bone appeared to be regenerating, whether the microstructures of the bone

and their mechanical capabilities improved could not be determined solely from the bone density or bone configuration, and the bone mineral density (BMD) often recovered prior to the BAp orientation. In fact, it has been reported that the degree of BAp orientation and BMD strongly influence the mechanical properties. Recently, a nanoindentation technique has been developed and widely used for measuring the microlevel mechanical properties of small, thin, and microstructured materials [9]. This technique can be used to determine mechanical properties such as the Young's modulus, hardness, and viscoelasticity of regenerated bone [10]. The hardness and Young's modulus of the regenerated bone are found to be significantly lower than those of the intact bone.

In actuality, BMD recovers faster than the BAp orientation; the recovery rate of BAp orientation is initially slow, following which it accelerates during bone regeneration in defective parts. Thus, an analysis of the controlling factors of the bone microstructure during regeneration shows the BAp alignment to be far more important than the BMD.

3 Design of Materials for Promoting Bone Regeneration by Taking into Account BAp Alignment

3.1 Biomaterial Design Based on Bone Anisotropy

Because the bone microstructure is anisotropic, materials meant to serve as a substitute for bone should be developed based on the anisotropy. Materials for stimulating the BAp alignment are designed according to the degree of BAp alignment in the bone. The shape and structure of biomaterials should be optimally designed.

For bone replacement materials, biomedical metals are often used because they are mechanically safe and reliable. However, they pose problems of bone reabsorption and deterioration due to the effects of stress shielding to the bones. As a countermeasure, β -titanium alloys are used to lower the degree of elasticity [11]. From the viewpoint of the structural anisotropy of bone, by using directional porous metals [12] and by making use of the anisotropy of elongated pores, the bone regeneration can be controlled [13].

The porous implants were implanted such that the pore direction was parallel or perpendicular to the mesiodistal axis of the mandible (Fig. 4) [13]. A model of a lotus-type porous implant was used to understand the effect of the unidirectional elongated pore direction, either parallel or perpendicular to the mesiodistal direction, on the bone regeneration in an anisotropic bone tissue of a beagle mandible. As shown in Fig. 4 (lower figure), the degree of calcification and the subsequent preferred degree of the BAp *c*-axis orientation are higher in the elongated pores parallel to the mesiodistal axis than in the pores perpendicular to the axis, indicating that the formation of new calcified bone strongly depends on the elongated pore direction in the mandible, which has an anisotropic microstructure. This indicates that the induction

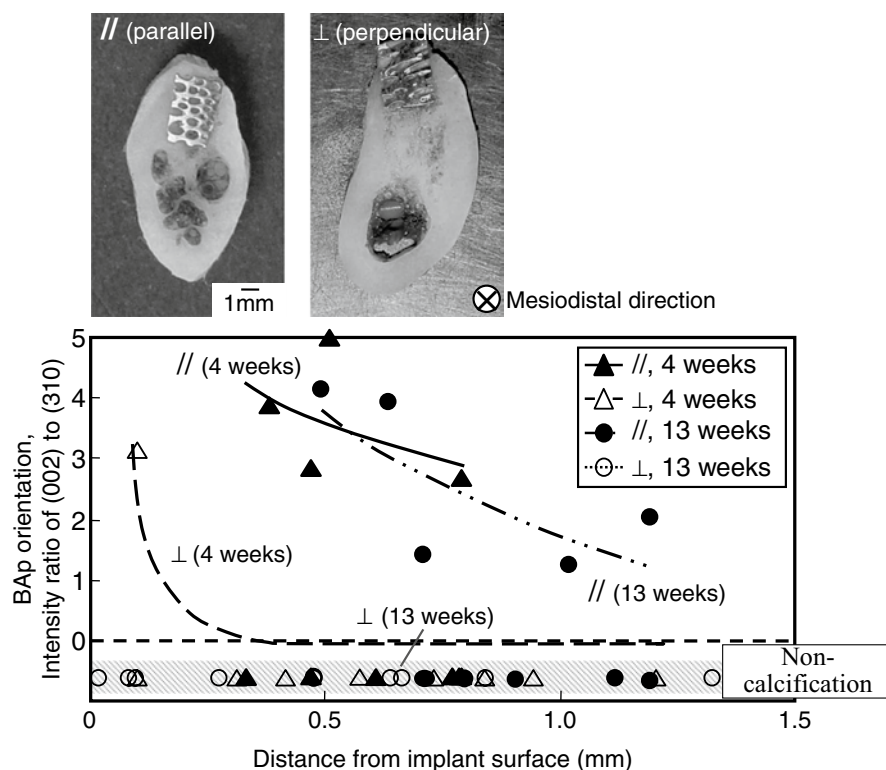


Fig. 4. Relationship between the intensity ratio of (002)/(310) and the distance from the porous implant surface after implantation for 4 and 13 weeks. The intensity ratio is determined in the calcified portion. Because non-calcified tissue does not show diffraction peaks from the calcium phosphates containing BAp, the data are plotted in the hatched area under zero of the intensity ratio. “//” and “⊥” indicate that the elongated pores in the implants were fixed parallel and perpendicular to the mesiodistal direction, respectively. This figure was redrawn from [13]

of elongated pores along the preferential alignment of the BAp/collagen complex in the original tissues represents remarkable regeneration in terms of bone volume and quality. Thus, when designing implants, it is necessary to evaluate the quality of the bone surrounding the implant and to take into account the anisotropy of bone microstructures in order to improve the regeneration process [13, 14].

3.2 Metal Implant Containing Anisotropic Open Pores Fabricated by Electron Beam Melting Method

Some rapid prototyping techniques have been developed for fabricating arbitrarily shaped nonporous and porous materials. The materials are produced in cross-sectional layers using a three-dimensional (3D) computer model [15]. The typical process of

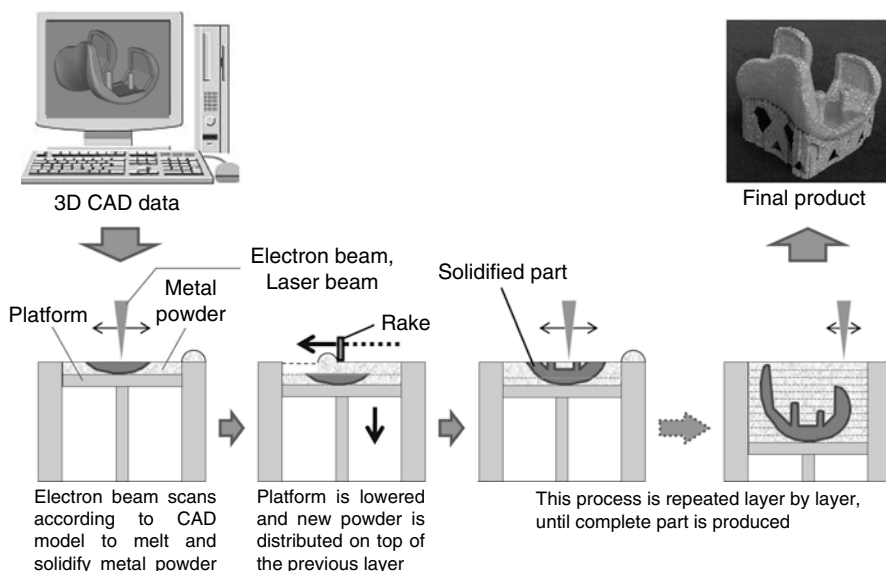


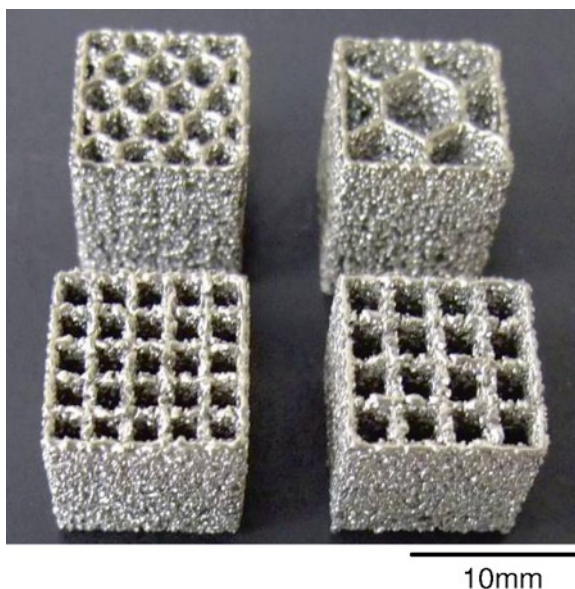
Fig. 5. Schematic figure showing the electron beam melting (EBM) or selective laser sintering process. The starting materials are metal powders, and the product with arbitrary morphology can be obtained without the use of a casting mould

the electron or selective laser sintering method is schematically shown in Fig. 5. The starting materials are metal powders, and the desired product is fabricated layer-by-layer. Figure 6 shows an example of metal implants containing anisotropic open pores fabricated by the electron beam melting (EBM) method [16].

New cylindrical bone implants containing elongated pores interconnected as open pores were also fabricated by the EBM method using Ti-6 mass% Al-4 mass% V ELI powder as a starting material (Fig. 7a, b). Thus, unidirectional open pores were introduced in the direction of the cylindrical axis, and the pores were also connected in the vertical direction. In contrast, the defective portion was not covered with new bone in the absence of the new implant. The unidirectional porous implant connected the defective part with new bone by forming a bone-marrow cavity at the center and cortical bones in the surrounding area (Fig. 7c–e) [17].

New bone formation in the elongated pores of the implant and the preferential degree of the BAp *c*-axis orientation were confirmed along the long bone axis by microbeam X-ray diffraction (Fig. 7f). The bone mass and preferential orientation of the BAp *c*-axis, which were considered as parameters determining the bone quality, decreased with the distance from the edge of the implant along the longitudinal bone axis because of a weak stress-shielding effect. Nevertheless, there was no bone absorption even after 24 weeks, and the BAp *c*-axis orientation along the bone longitudinal axis was observed even at the center of the implant, albeit to a lesser extent.

Fig. 6. Appearance of porous Ti-6Al-4V alloy products fabricated by the EBM method. The *upper* and *lower* images show the products containing elongated pores with a honeycomb and square cross section, respectively



Bone regeneration was conducted in the implant grafted in the defects of rabbit ulna, with a focus on the bone mass and bone quality of new bones. This finding may be attributed to the fact that this implant has open pores not only in the direction of the cylindrical axis but also in the vertical direction, and this is favorable for bone-marrow fluid flow or cell migration.

4 Conclusion

This study focuses on the orientation degree of the BAp *c*-axis, and it is found that the microstructure of bones changes during bone regeneration and new bone formation with or without implants. It is necessary to consider the anisotropy of the bone microstructure when designing materials meant as substitutes for bone. Biomaterials for tissue generation should be developed and designed by taking into account both the material and biological properties; therefore, it is expected that future studies will encompass various fields such as engineering, medicine, dentistry, and biology.

In the future, we intend to clarify the formation mechanism of the BAp preferential alignment and to control the degree of BAp orientation by using an anisotropic biomaterial design in order to realize a suitable distribution of the BAp *c*-axis orientation.

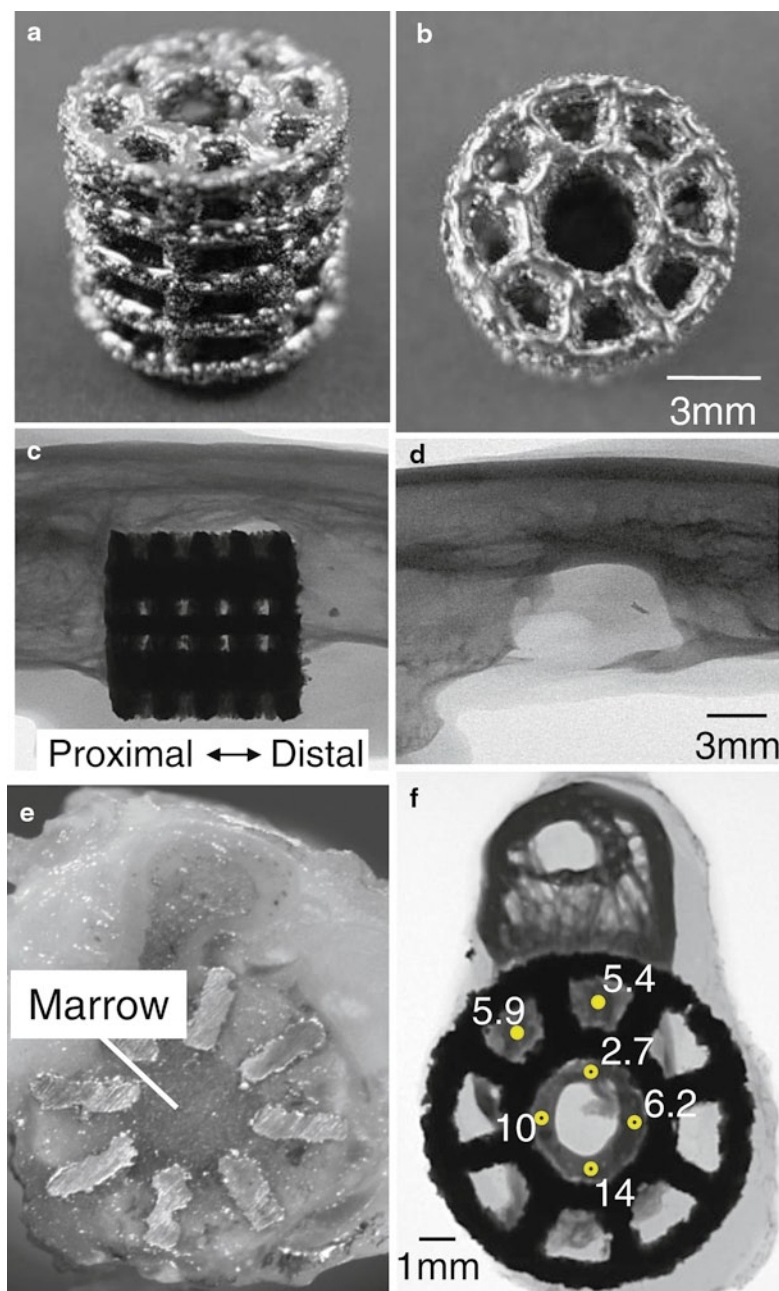


Fig. 7. Morphology of a cylindrical implant with elongated open pores fabricated by the EBM method (a, b). Radiographs of defected rabbit ulnar portion with the implant at 24 weeks after operation (c) and defected portion without the implant at 24 weeks (d). Formation of bone marrow can be seen in the implant (e). Moreover, the preferential degree of BAp orientation parallel to mesiodistal direction highly recovers compared to the degree of 2 in randomly oriented apatite (f). This figure was redrawn from [17]

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The Development of Binary Titanium Alloys with the Aim of Dental Applications

Yukyo Takada, Masatoshi Takahashi, and Masafumi Kikuchi

Abstract. The effects of the β -stabilizing elements (Ag, Au, Co, Cr, Cu, Fe, Mn, and Pd) on the characteristics of the experimental binary titanium alloys were examined with respect to mechanical properties, high temperature reactivity, corrosion resistance, grindability and machinability. The addition of β stabilizers to titanium lowers the fusion temperature, improves working mechanical properties, and suppresses reactivity at high temperature while maintaining corrosion resistance. The Ti–Cr or Ti–Mn alloys display similar favorable properties as dental casting titanium alloys. On the other hand, the Ti–Ag alloys may belong to the free-cutting materials, and demonstrate inhibitory properties on biofilm formation without affecting normal microbial flora. The Ti-20mass%Ag alloy is especially noted for its potential use as a dental CAD/CAM alloy, which may be potentially used not only for dental use but also for other medical applications.

Key words. β -Stabilizing element, Bacteriostatic biomaterials, Corrosion resistance, Machinability, Mechanical property

1 Introduction

Although titanium has been well documented and used for many dental applications, its use requires several technical hurdles to be overcome, in order to be used as easily as conventional dental metals. Titanium has a disadvantage in that conventional dental alloy casting systems and schedules cannot be used because of the titanium's high fusion temperature and also its reactivity at high temperature.

Y. Takada (✉), M. Takahashi, and M. Kikuchi
Division of Dental Biomaterials, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: takada@m.tohoku.ac.jp

Recently, CAD/CAM machining has been used to fabricate titanium dental devices. However, titanium is one considered a more difficult material to machine cut, and there is a relative negative effect based upon increased cutting time and reduced cutting tool longevity. The alloying of titanium with beta-stabilizing elements is expected to resolve many of the material disadvantages of titanium that are not optimized for dental casting or machining.

The authors have examined the effects of the several beta-stabilizing elements on the characteristics of experimental binary titanium alloys. The potential for new titanium alloys incorporating these elements is discussed and examined by testing and evaluation of the mechanical properties, and also its high temperature reactivity, corrosion resistance, and machinability. Ti–Ag alloys are introduced as a new free-cutting material with a biofilm inhibitory property, useful for dental applications that use CAD/CAM dental device machining technology.

2 Materials and Methods

Eight elements such as Ag, Au, Co, Cr, Cu, Fe, Mn, and Pd were chosen with respect to giving a low fusion temperature, a wide β region and a low eutectoid temperature efficiently. The binary titanium alloys together with the beta-stabilizing elements were prepared using an argon arc melting furnace.

The experimental binary titanium alloys were evaluated using X-ray diffraction spectroscopy for observation of microstructures, tensile testing for strength evaluation, and evaluative electrochemical measurements. Physical property grinding and machining tests were performed using a carborundum wheel and a CAM system equipped with a $\phi 3$ mm square end mill, respectively. A biofilm surface formation test was utilized for the Ti–Ag alloys. *Streptococcus mutans* (*Sm*) ATCC 31989 was cultured anaerobically in a complex medium in the presence of each specimen. The specimen accumulated bacteria formations were then evaluated. In addition, the bactericidal activity of these metal plates was also evaluated according to the JIS Z 2801 using *Sm* NCTC10449.

3 Fusion Temperatures and Alloy Phases

All selected elements demonstrated a reduction in the alloy fusion temperature as the element content was increased. A descending order of fusion temperature reducing effect is as follows: Cu, Co, Fe, Mn, Pd, Cr, Au and Ag. The addition of Co, Cu, Fe and Mn demonstrated a reduction of the titanium alloy fusion temperature to below 1,300°C by the addition of 20 mass%.

Differential alloy phase changes between the different added elements resulted in the categorization of these elements into three main groups. The phases transformed from α to α +intermetallic compounds (Group 1), from α to β by way of α + β (Group 2), and from α to β +intermetallic compounds by way of α + β and β (Group 3) as the β stabilizer contents increased to 30 mass% (Table 1). Ag, Au, and

Table 1. Phases of the binary titanium alloys with the β stabilizers

Group/ element	Compositions (mass%)						
	5	10	15	20	25	30	40/45
1 Au	α	α		α			$\alpha + \text{Ti}_3\text{Au}$
	Ag	α	α	α	$\alpha + \text{Ti}_2\text{Ag}$	$\alpha + \text{Ti}_2\text{Ag}$	$\alpha + \text{Ti}_2\text{Ag} + \text{TiAg}$
	Cu	$\alpha + \text{Ti}_2\text{Cu}$	$\alpha + \text{Ti}_2\text{Cu}$	$\alpha + \text{Ti}_2\text{Cu}$		$\alpha + \text{Ti}_2\text{Cu}$	
2 Cr	$\alpha + \beta$	β	β	β		β	
	Mn	$\alpha + \beta$	β	β		β	
3 Co	$\alpha + \beta$	β	$\beta + \text{Ti}_2\text{Co}$	$\beta + \text{Ti}_2\text{Co}$		$\beta + \text{Ti}_2\text{Co}$	
	Fe	$\alpha + \beta$	β	β		$\beta + \text{TiFe}$	
	Pd	$\alpha + \beta$	$\alpha + \beta$	$\alpha + \beta$	β	$\alpha + \text{Ti}_2\text{Pd} + \text{TiPd}_2$	

Cu belonged to the group 1 and could not retain β phase because of high eutectoid temperature. However, the other elements could retain meta-stable β phase at room temperature [1].

4 Mechanical Properties

The tensile strength and elongation of the Ti–Cr alloys increased and reached to a maximum value when their phases changed to the single β phase as the Cr content increased. On the other hand, their alloyed hardness decreased and showed a minimum value in the single β phase. The β phase substantially contributes to an improvement of the mechanical properties. The changes of the mechanical properties of the other alloys were similar to those of the Ti–Cr alloys.

The strength and elongation of the alloys with the single α or β phase were summarized in Fig. 1. The strengths of all alloys are larger than that of titanium. The β and α phase alloys had large strength and elongation, respectively. In the case of Cr, Fe and Mn, the strengths were 1,000 MPa or more with maintenance of their elongation properties. The Ti-20%Ag alloy showed about 550 MPa with elongation of 20% despite the single α phase [2].

5 Reactivity at High Temperature

The binary titanium alloys were cast in a phosphate bonded investment molds. Hardened layers over 100 μm depth were seen. Reaction between the alloys and the investment mold led these hardened layers. Reactivity of the alloys was evaluated by ratios of hardness in depth of 20 μm (reactive area) and 500 μm (unreactive area). Adding β stabilizers over 10 mass% reduced hardness at the surface to 1.1–2 times of that at the bulk. The areas in depth of 20 μm and 500 μm from the surface were analyzed by EPMA. The distribution of O and Si tended to decrease. Alloying with β stabilizers suppresses to react with O and Si.

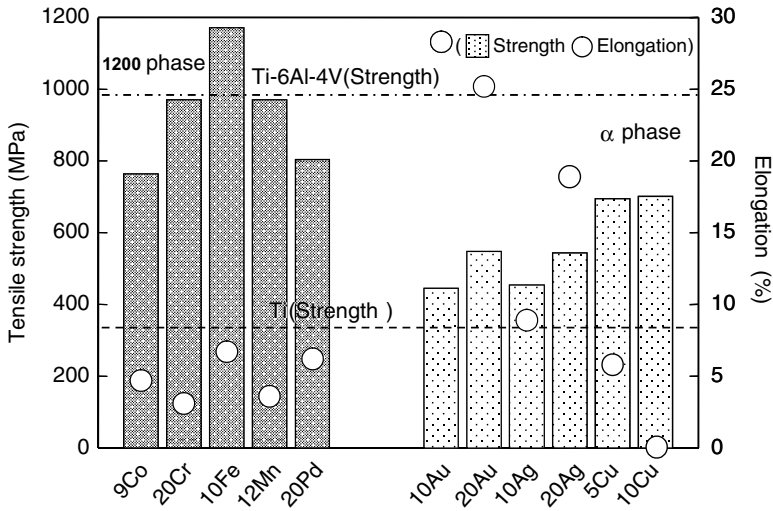


Fig. 1. Mechanical properties of the binary titanium alloys with the α or β phase

6 Corrosion Resistance

In the case of the alloys composed of solid solution of the α or β phase, and hardly-soluble intermetallic compounds, the profiles of anodic polarization curves were similar to that of titanium. The precipitated intermetallic compounds did not change before and after potentiostatic anodic polarization [1]. Addition of Ag over 20 mass% caused the soluble intermetallic compounds of Ti_2Ag or $TiAg$. The precipitated intermetallic compounds preferentially dissolved and decreased corrosion resistance. Corrosion resistance of the alloys composed of the solid solution phases and the hardly-soluble intermetallic compounds does not diminish even when alloyed. However, the corrosion resistance of alloys with the soluble intermetallic compounds depends on their individual solubilities.

7 Grindability

After the experimental binary titanium alloys with Ag, Au, Cu, Hf and Nb were prepared using an argon arc melting furnace, a grinding test to demonstrate the working manipulation properties was performed for these alloys. The alloying of titanium with Au and Hf did not demonstrate improved grindability. However, a Ti-20mass%Ag alloy showed a higher ease of grindability, about 2.5 times than that of titanium [3].

8 Effects of β Stabilizers

The alloying of titanium with β stabilizers can add the several functional property improvements. The alloy addition of β stabilizers to titanium lowers the fusion temperature, adds improved mechanical properties, and suppresses reactivity at high temperature while maintaining corrosion resistance. Although not currently used for dental treatments, Ti–Cr or Ti–Mn alloys display similar favorable properties as dental casting titanium alloys. Ti–Ag alloys appear to have potential as a free-cutting materials.

9 Free-Cutting Materials

The Ti-20mass%Ag alloy was selected for the machining test because of its good grindability. The cutting force required for Ti-20mass%Ag alloy was about 80% that of pure titanium metal [4]. The resulting cut surfaces were smooth and cutting tool life was found to be longer those tools used for titanium cutting. The Ti-20mass%Ag alloy may belong to the free-cutting materials.

10 Bacterial Activity and Biofilm Formation

The inhibitory activity on the biofilm formation and the bactericidal activity in the Ti–Ag alloys were examined. The biofilm amount on the Ti–Ag alloys with 20 and 25mass%Ag was significantly lower ($p < 0.05$) than those on pure titanium, pure silver, and an Au–Ag–Pd alloy [5]. In addition, the Ti–Ag alloys as well as pure titanium showed no bactericidal activity. The Ti–Ag alloys are mildly antimicrobial or bacteriostatic biomaterials, which may demonstrate inhibitory properties on biofilm formation without affecting normal microbial flora.

11 Conclusion

The alloying of titanium with the beta-stabilizing elements have shown improved efficacy by reduction of fusion temperatures, improved working mechanical properties and better reactivity with a maintenance of corrosion resistance. The Ti-20mass%Ag alloy is especially noted for its potential use as a dental CAD/CAM alloy, which may be potentially used not only for dental use but also for other medical applications.

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π -Phase and χ -Phase: New Precipitates in Biomedical Co–Cr–Mo Alloys

Takayuki Narushima, Shingo Mineta, Alfirano, and Kyosuke Ueda

Abstract. The phases of precipitates in ASTM F75 and F799 Co–Cr–Mo alloys are reviewed with a focus on new precipitates of the π - and χ -phases. The π -phase carbide was observed at temperatures of 1,548–1,623 K where partial melting in Co–Cr–Mo–C system alloys occurred. Si was slightly enriched in the π -phase precipitate and nitrogen substituted carbon in the octahedral sites of the π -phase. It is suggested that the formation and stability in the partial melting region and the transformation of the π -phase take part in the final constitution of the precipitates in ASTM F75 and F799 alloys. A χ -phase has been detected in the as-cast and heat-treated Co–Cr–Mo alloy with a midlevel carbon content and the addition of Si. The χ -phase precipitate is an intermetallic compound. The conditions under which the χ -phase is formed are limited by the heat-treatment temperature and chemical composition.

Key words. χ -phase, π -phase, ASTM F 75, Co–Cr alloy, Precipitation

1 Introduction

Co–Cr alloys have been applied as materials for orthopedic, cardiovascular, and dental wires due to their attractive properties such as excellent mechanical properties, high corrosion resistance, and high wear resistance [1, 2]. In particular, ASTM F75 and F799 Co–Cr–Mo alloys have been recognized as effective Ni-free Co–Cr alloys and have been widely used for sliding parts in artificial joints. In addition, since the development of Vitallium in the 1930s, they have a long history of practical applications in dental and medical fields [3, 4].

T. Narushima (✉), S. Mineta, Alfirano, and K. Ueda
Department of Materials Processing, Tohoku University,
6-6-2 Aoba, Aramaki, Aoba-ku, Sendai 980-8579, Japan
e-mail: narut@material.tohoku.ac.jp

Precipitates in Co–Cr–Mo alloys are known to affect the properties of both cast and wrought products. Cast Co–Cr–Mo alloys are used to manufacture biomedical devices such as artificial joints and denture bases because of their high wear resistance, which is attributed to strength imparted by carbides formed in the alloy. For wrought Co–Cr–Mo alloys, carbide precipitation is suppressed to improve their hot workability [5]. Therefore, the knowledge of the phase, size, amount, and distribution of precipitates under as-cast condition and after heat treatment is essential to establish reasonable production processes for biomedical Co–Cr–Mo alloy devices.

In this review, the phases of precipitates observed in biomedical Co–Cr–Mo–C–N–Si–Mn alloys are discussed based on research by the present authors. Since ASTM F75 and F799 Co–Cr–Mo alloys have an almost constant content of Cr (≈ 28 mass%) and Mo (≈ 6 mass%), the effects of the carbon, nitrogen, Si, and Mn content on the precipitates are focused on. Additionally, new π -phase [6, 7] and χ -phase [8] precipitates are introduced. Hereafter, the chemical composition of the alloys is in units of mass% although the mass% notation is omitted.

2 Phases of Precipitates

$M_{23}C_6$ type and η -phase (M_6C – $M_{12}C$ type) carbides (M refers to metallic elements such as Cr) and intermetallic σ -phase (Co(Cr,Mo)) are well-known precipitates in ASTM F 75 and F 799 Co–Cr–Mo alloys [9–11]. The precipitation of an M_7C_3 type carbide was suggested in biomedical Co–Cr–Mo alloys [12]. An M_2N type nitride was reported to be formed from a eutectoid transformation, $\gamma \rightarrow \varepsilon + M_2N$, during the aging of a Co-27Cr-5Mo-0.04C-0.16N alloy at 1,073 K for 90 ks [13]. Recently, the present authors revealed the first report of π -phase [6, 7] and χ -phase [8] precipitates in biomedical Co–Cr–Mo alloys.

3 π -Phase Precipitate

Figure 1 shows the XRD patterns of precipitates electrolytically extracted from as-cast Co–Cr–Mo alloys with carbon contents of 0.12, 0.16, 0.26, and 0.35 mass% [6]. σ -phase and $M_{23}C_6$ type precipitates were the main precipitates in alloys with low and high carbon contents, respectively. The XRD pattern of the precipitates in Co-27.5Cr-6.0Mo-0.16C alloy was complicated and revealed the presence of another phase; the XRD reflections of this phase were in good agreement with the pattern of a $(Cr,Mo)_{12}(Fe,Ni)_{8-x}N_{4-z}$ type nitride (JCPDS card no. 26–0428), which was detected as a precipitate after aging of an Fe-25Cr-28Ni-2Mo-0.31N alloy [14]. This nitride phase is referred to as the π -phase and exhibits a β -Mn structure. Its chemical composition can be ideally represented by M_2T_3X , where M and T are metallic elements with a low and high affinity for X (i.e., carbon and nitrogen), respectively. Figure 2 depicts a crystallographic illustration of the π -phase. Carbon

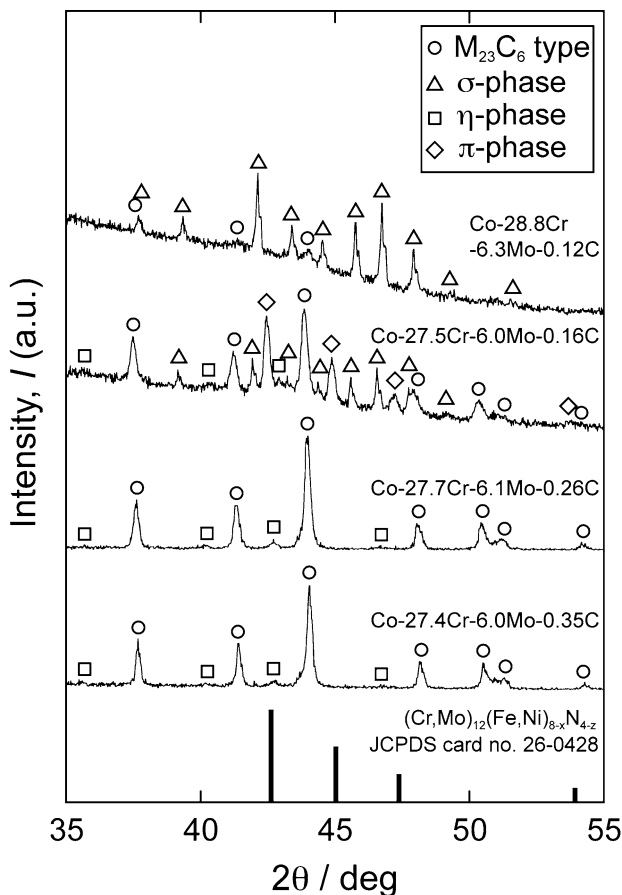


Fig. 1. XRD patterns of precipitates electrolytically extracted from Co–Cr–Mo alloys with carbon contents of 0.12–0.35 mass%

and nitrogen are introduced into the octahedral sites formed due to the high affinity of T for X.

Figure 3 summarizes the phases of precipitates formed during the heat treatment of a Co-28.1Cr-5.9Mo-0.24C alloy [15]. The formation of π -phase carbide was confirmed at temperatures of 1,548–1,623 K, but was not observed at lower temperatures. The π -phase was starlike-dense in appearance (Fig. 4). These results suggest that partial melting at the interdendritic parts and/or grain boundary is closely related to the formation of the π -phase. The π -phase is thermodynamically stable in the ASTM F 75 and F 799 compositional range at 1,573–1,623 K as it can be observed after heat treatment even for a relatively long holding time of 43.2 ks [15]. The presence of a π -phase precipitate in the alloys depends on the cooling rate of the casting process. The rapid cooling might be required for the remaining of π -phase precipitate in the alloys. The lower cooling rate seems to cause the transformation of the π -phase

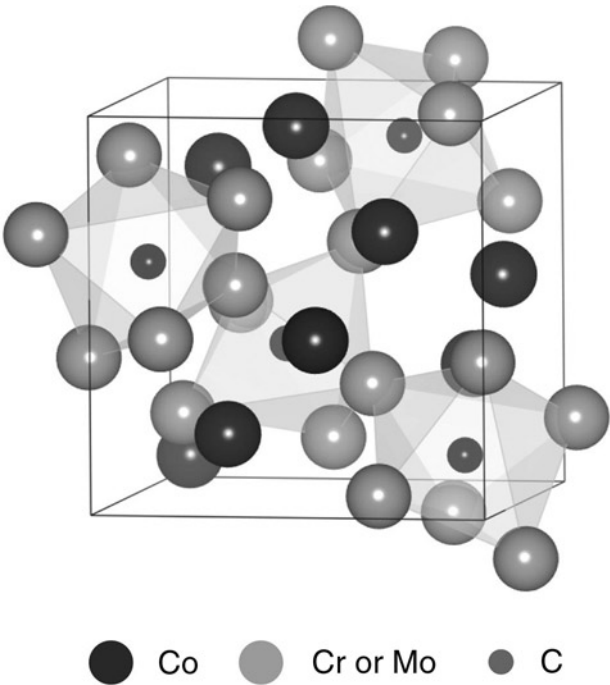


Fig. 2. Schematic representation of the crystallographic structure of the π -phase with a chemical composition of M_2T_3X . M (Co) and T (Cr or Mo) are metallic elements with low and high affinity for X (i.e., carbon and nitrogen), respectively

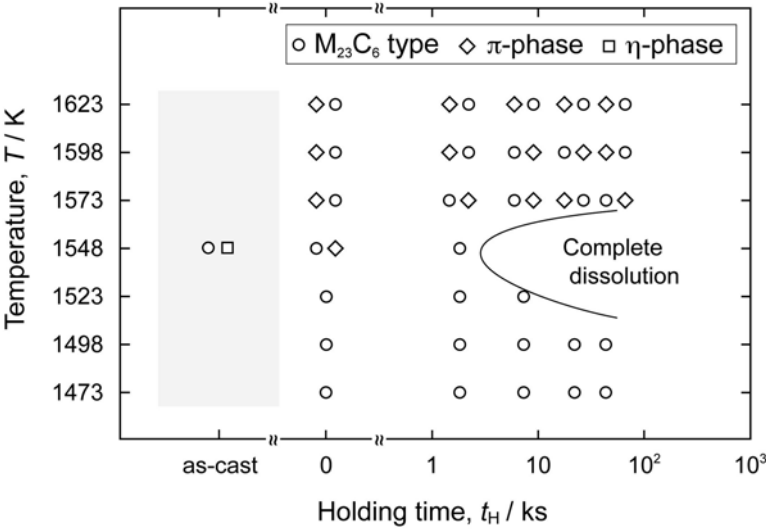


Fig. 3. Types of precipitates in as-cast and heat-treated Co-28.1Cr-5.9Mo-0.24C alloy

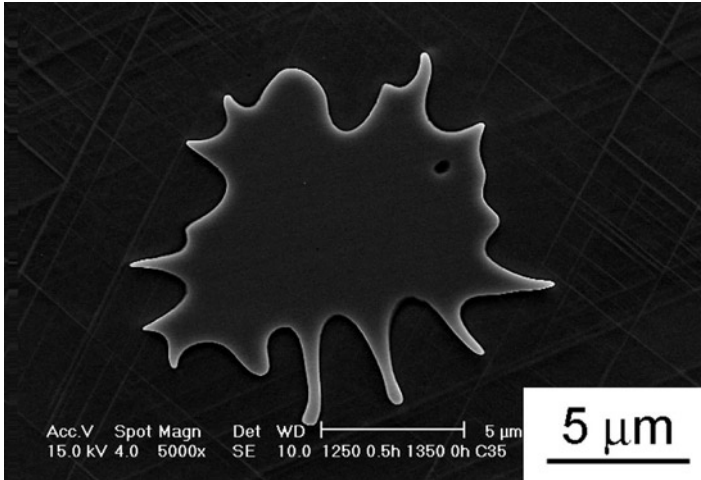


Fig. 4. π -phase precipitate with a starlike-dense appearance in Co-27.4Cr-6.0Mo-0.35C alloy

Table 1. Chemical composition of π -phase precipitate in Co-27.7Cr-6.0Mo-0.24C-0.17N-1.2Si-1.2Mn alloy heat treated at 1,548 K for 0 ks (mass%)

Co	Cr	Mo	C	N	Si	Mn
34.4	38.1	21.7	2.7	0.7	1.3	1.1

to other phases such as $M_{23}C_6$ type carbide + γ -phase. The formation and transformation of π -phase appear to take part in the final constitution of the precipitates in Co–Cr–Mo alloys because the casting process incorporates the partial melting region in which the π -phase precipitate is presumed to form.

Table 1 shows the chemical composition of the π -phase detected in Co-27.7Cr-6.0Mo-0.24C-0.17N-1.2Si-1.2Mn alloy heat treated at 1,548 K for 0 ks [8]. Si was slightly enriched in the π -phase precipitate and nitrogen substituted carbon up to 0.7 mass% (2.7 at%) in the octahedral sites of the π -phase. Both Si and nitrogen were determined to stabilize the π -phase.

4 χ -Phase Precipitate

The presence of χ -phase precipitate was confirmed in the as-cast and heat-treated Co-27.1Cr-5.6Mo-0.13C-1.3Si alloys. The XRD pattern of the precipitate electrolytically extracted from the as-cast alloy is shown in Fig. 5 along with JCPDS card no. 032–0637 (α -Mn). The χ -phase exhibits a body-centered cubic (bcc) structure and has an A12 (α -Mn) structure with 58 metallic atoms per unit cell (Fig. 6).

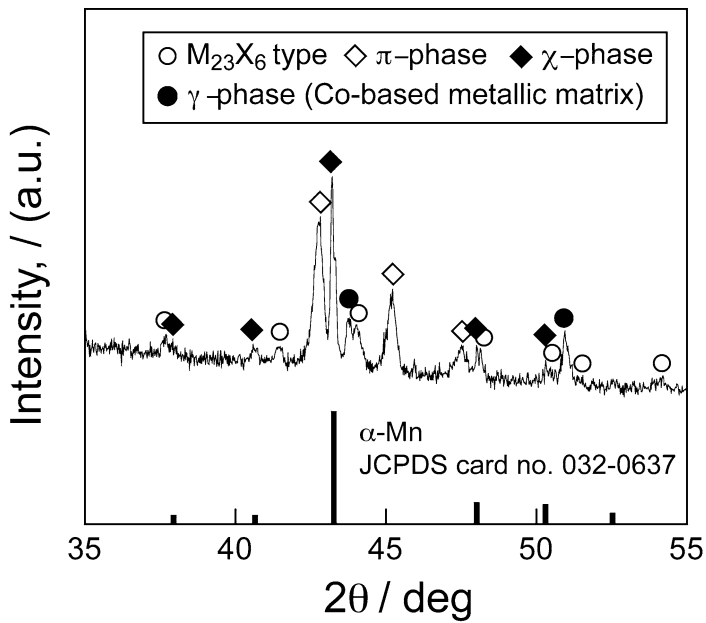


Fig. 5. XRD pattern of the precipitate electrolytically extracted from as-cast Co-27.1Cr-5.6Mo-0.13C-1.3Si alloy

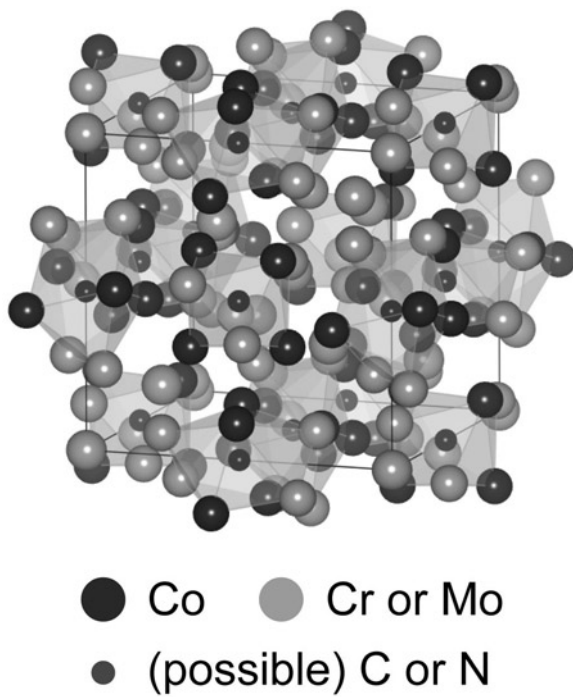


Fig. 6. Schematic representation of the crystallographic structure of the χ -phase. Possible site of carbon or nitrogen is shown

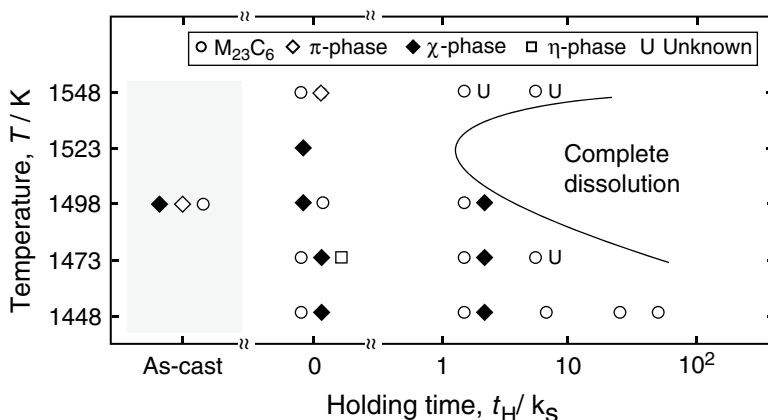


Fig. 7. Phase of precipitates in as-cast and heat-treated Co-27.1Cr-5.6Mo-0.13C-1.3Si alloy

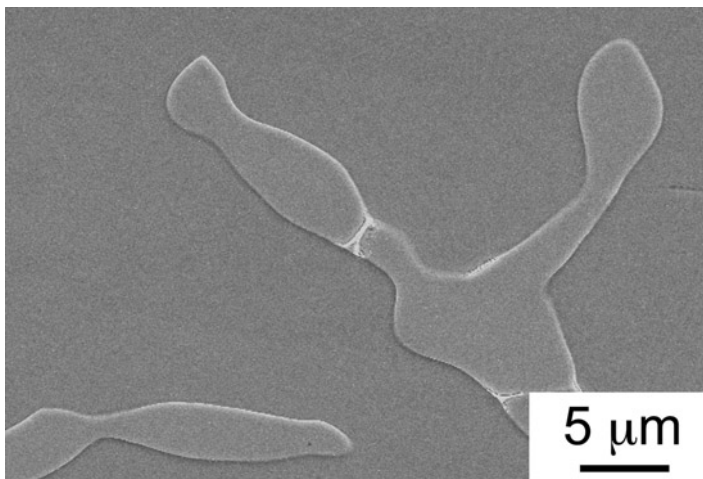


Fig. 8. χ -phase precipitate with a blocky-dense appearance in Co-27.1Cr-5.6Mo-0.13C-1.3Si alloy heat treated at 1,523 K for 0 ks

Figure 7 summarizes the phases of precipitates in the as-cast and heat-treated Co-27.1Cr-5.6Mo-0.13C-1.3Si alloy. A single χ -phase precipitation region was detected after heat treatment at 1,523 K for a holding time of 0 ks. The SEM image and chemical composition of the χ -phase precipitate are shown in Fig. 8 and Table 2, respectively. The χ -phase precipitate was blocky-dense in appearance and was Si-enriched.

The presence of the χ -phase as a precipitate was previously reported in Fe-based alloys after isothermal heat treatment [16, 17]. This χ -phase precipitate was

Table 2. Chemical composition of χ -phase precipitate in Co-27.1Cr-5.6Mo-0.13C-1.3Si alloy heat treated at 1,473 K for 1.8 ks (mass%)

Co	Cr	Mo	C	Si
47.7	30.0	20.3	^a	2.0

^aAlmost the same as carbon content in metallic matrix

recognized as an intermetallic compound. On the other hand, Goldschmidt suggested that carbon could dissolve in the χ -phase up to ~5 at%, i.e., 3 interstitial carbon atoms in 58 metallic atoms in the unit cell [18]. In this study, FE-EPMA analysis revealed no significant enrichment of carbon in the χ -phase precipitate as compared with the Co based metallic matrix (γ -phase). Therefore, it can be concluded that the χ -phase precipitate in the Co–Cr–Mo alloy is an intermetallic compound. A possible carbon or nitrogen site is shown in Fig. 6 but the amount of carbon contained in these sites of the Co–Cr–Mo–C–Si system alloy seems to be small.

The formation of the χ -phase requires an alloy composition with midlevel carbon content and the addition of Si, e.g., Co-27.1Cr-5.6Mo-0.13C-1.3Si.

The χ -phase precipitate is considered to be harmful in Fe-based alloys because it is deleterious to their mechanical properties. In Co–Cr–Mo alloys, the conditions under which the χ -phase is formed are limited by the heat-treatment period and chemical composition. Therefore, it is relatively easy to prevent the formation of the χ -phase during the fabrication of Co–Cr–Mo alloy implants.

5 Conclusion

It was surprising that new π -phase and χ -phase precipitates were found in biomedical Co–Cr–Mo alloys, which have a long history of successful application in implants. The presence of various types of precipitates and the complexity of their structures are the main reasons why the π - and χ -phases had not been detected before our studies. The formation in the partial melting region at 1,548–1,623 K and the transformation during cooling process of the π -phase take part in the final constitution of precipitates. The intermetallic χ -phase precipitate was observed in the Co–Cr–Mo alloy with midlevel carbon content and the addition of Si but conditions suitable to the formation of the χ -phase were limited.

The future work on the precipitates includes: (a) the relationship between the π -phase and other phases at high temperatures and (b) the effects of the π - and χ -phase precipitates on the properties of Co–Cr–Mo alloys such as mechanical strength, ductility, and corrosion and wear resistances. These studies enable the selection of the types, size, amount, and distribution of precipitates in Co–Cr–Mo alloys via addition of alloying elements and heat treatments to optimize alloys for their applications.

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Session I
Biomechanical–Biological Interface

Classification of Chewing Cycles: Different Motion Paths May Indicate Differences in Function

Yohei Igari, Yuko Komine, Yasue Tanaka, Mai Sato,
Alexander Wirianski, and Yoshinori Hattori

Abstract. Chewing cycles of ten healthy adults who ingested cooked rice were classified into three groups by using principal component and cluster analyses. The appearance ratios of the groups differed significantly among the stages, when chewing sequences were equally divided into three stages according to the number of cycles (paired-*t* test with Bonferroni correction, $p < 0.016$). This suggests that groups which differ in form may also differ in function.

Key words. Chewing-cycle trajectory, Cluster analysis, Principal component analysis

1 Introduction

Chewing comprises several groups of motion which differ either in form and/or in function. It is unclear whether or not the groups which differ in form also differ in function. The purpose of this study was to (1) develop a method of classifying

Y. Igari (✉), Y. Komine, Y. Tanaka, and Y. Hattori
Division of Aging and Geriatric Dentistry, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai, Japan
e-mail: igariyohei@hotmail.co.jp

M. Sato
Division of Physical Medicine and Rehabilitation, Tohoku University
Graduate School of Medicine, Sendai, Japan

A. Wirianski
Division of Aging and Geriatric Dentistry, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai, Japan
and
Jaw Function and Orofacial Pain Research Unit, Faculty of Dentistry,
The University of Sydney, Sydney, NSW, Australia

chewing cycles based on their form, and; (2) compare the appearance ratio of the classified groups among the different stages of the chewing sequence.

2 Methods

Three-dimensional mandibular motion paths were registered during ten chewing sequences in ten healthy adults who chewed a standardized 10-g mouthful of cooked rice on their preferred sides until swallowing. Each chewing cycle path was described using the 3D-coordinates of the jaw at time points which equally divided the opening and closing phases of the cycle into 20 intervals. Intra-individual variance of the chewing paths was evaluated using principal component (PC) analysis [1] and classified into three groups using cluster analysis based on the PC scores that accounted for >90% of the variance. Chewing sequences were equally divided into three stages according to the number of cycles. Appearance ratios of the groups were compared between the stages.

3 Results and Discussion

In all subjects, the appearance ratios of the classified groups, as well as the size and shape characteristics (mean PC scores) within the groups, differed significantly among the stages (paired-*t* test with Bonferroni correction, $p < 0.016$) (Fig. 1). This suggests that groups which differ in form may also differ in function.

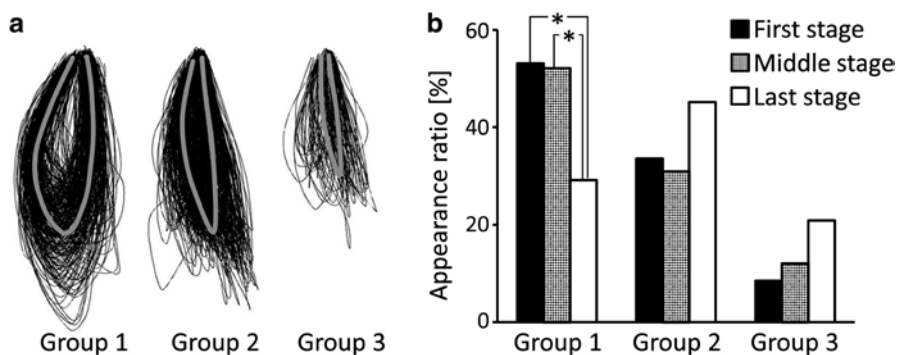


Fig. 1. Classified groups of chewing cycles (a) and their appearance ratio (b) (Subject 1). (a) Chewing cycles were classified by using principal component and cluster analyses. *Thin black and thick gray lines* show all and average chewing cycles in the coronal plane, respectively. Each group contains chewing cycles with similar size and shape characteristics. (b) Appearance ratio of each group was compared between the different stages of the chewing sequences. *Asterisks* indicate significant difference between stages ($p < 0.016$)

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Bactericidal Effect of Hydroxyl Radical Generated by Photolysis of Hydrogen Peroxide

Hiroyo Ikai, Keisuke Nakamura, Midori Shirato, Taro Kanno,
Atsuo Iwasawa, Yoshimi Niwano, Keiichi Sasaki,
and Masahiro Kohno

Abstract. The relationship between the yield of hydroxyl radical generated by photolysis of H_2O_2 and its bactericidal effect was evaluated. To generate hydroxyl radical, H_2O_2 was irradiated with laser light at a wavelength of 405 nm. ESR spin trapping analysis showed that the yield of hydroxyl radical increased with either the laser irradiation time or the concentration of H_2O_2 . *Streptococcus mutans* and *Porphyromonas gingivalis* were used in the bactericidal assay. The assay showed that the bactericidal effect was related to the laser irradiation time and the concentration of H_2O_2 . Laser irradiation of the planktonic and the biofilm model of *S. mutans* in 1,000 mM H_2O_2 and *P. gingivalis* in 500 mM showed a >5-log reduction of viable counts within 3 min and 30 s, respectively. These results suggest that the bactericidal activity against oral pathogenic bacteria consisting of facultative and obligate anaerobes is dependent on the yield of hydroxyl radical.

Key words. Bactericidal effect, Biofilm, Hydrogen peroxide, Hydroxyl radical, Photolysis

H. Ikai (✉), M. Shirato, T. Kanno, and K. Sasaki
Division of Fixed Prosthodontics, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: ikai@m.tohoku.ac.jp

K. Nakamura and Y. Niwano
Laboratory for Redox Regulation, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

A. Iwasawa and M. Kohno
Department of Bioengineering, Tokyo Institute of Technology,
4259-G1-25 Nagatsuta-cho, Midori-ku, Yokohama 226-8502, Japan

1 Introduction

A novel disinfection technique in which artificially generated hydroxyl radical can kill bacteria has been developed in our laboratory. Although it is well known that UV light can effectively generate hydroxyl radical from H_2O_2 , UV radiation also causes damage on normal tissues as an adverse effect [1]. From the aspect of clinical application, therefore, visible blue light (>400 nm) has been applied as a light source for safety reason [2]. In this study, to generate hydroxyl radical, H_2O_2 was irradiated with laser light at a wavelength of 405 nm. The purpose of the present study was to evaluate the bactericidal effect of hydroxyl radical artificially generated by photolysis of H_2O_2 , and the yield of hydroxyl radical required to disinfect two species of typical oral pathogenic bacteria.

2 ESR Analysis of Hydroxyl Radicals Generated by Photolysis of H_2O_2

When 1,000 mM H_2O_2 was irradiated by a laser diode with a wavelength of 405 nm, DMPO-OH was detected by ESR. The yield of DMPO-OH increased linearly with the laser irradiation time and the concentration of H_2O_2 .

3 Relationship Between the Yield of Hydroxyl Radical and the Bactericidal Effect

The bactericidal test was conducted planktonic and biofilm model by using two species of oral bacteria, *Streptococcus mutans* and *Porphyromonas gingivalis*. The result showed that the bactericidal effect was related to the laser irradiation time and the concentration of H_2O_2 . Laser irradiation of both the suspension and the biofilm of *S. mutans* in 1,000 mM H_2O_2 showed a >5-log reduction of viable counts within 3 min. In the case of the planktonic and the biofilm model of *P. gingivalis*, a >5-log reduction of viable counts was obtained only within 30 s when even 500 mM H_2O_2 was irradiated with the laser light. According to the ESR analysis of DMPO-OH, it is suggested that treatment of *S. mutans* with about 270 μM hydroxyl radical is enough to kill 99.999% of the bacterial cells. Similarly, the treatment of *P. gingivalis* with about 40 μM hydroxyl radical would result in a >5-log reduction of viable counts.

4 Conclusion

1. This disinfection method is very effective against the two species of oral pathogenic bacteria even in the biofilm model.
2. The bactericidal activity against oral pathogenic bacteria consisting of facultative and obligate anaerobes is dependent on the yield of hydroxyl radical.

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Comparison of Different Analytical Methods for Determining Singlet Oxygen Photogenerated from Rose Bengal

Kirika Ishiyama, Keisuke Nakamura, Hiroyo Ikai, Taro Kanno, Keiichi Sasaki, Yoshimi Niwano, and Masahiro Kohno

Abstract. Recently, antimicrobial effect of photodynamic therapy (PDT), which has originally been developed for cancer treatment, gets to be applied to dentistry. In PDT, singlet oxygen is generated by laser irradiation of photosensitizer to kill pathogens. However, an appropriate method to evaluate the yield of singlet oxygen generated in PDT has not been established. The present article discusses an appropriate way to evaluate the yield of singlet oxygen in PDT.

Key words. 1,3-diphenylisobenzofuran (DPIBF), Electron spin resonance, Fluorescent probe, Rose bengal, Singlet oxygen

1 Introduction

It is well known that singlet oxygen, one of reactive oxygen species, is highly oxidative and exerts strong cytotoxic effects. The cytotoxicity of singlet oxygen is applied for cancer treatment and antimicrobial therapy, which is known as photodynamic therapy (PDT). In dentistry, recent clinical data suggest a potential benefit of

K. Ishiyama (✉), H. Ikai, T. Kanno, and K. Sasaki
Division of Fixed Prosthodontics, Department of Restorative Dentistry,
Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku,
Sendai 980-8575, Japan
e-mail: kirika@dent.tohoku.ac.jp

K. Nakamura and Y. Niwano
Laboratory for Redox Regulation, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

M. Kohno
New Industry Creation Hatchery Center, Tohoku University,
6-6-10 Aoba, Aramaki, Aoba-ku, Sendai 980-8579, Japan

PDT in the treatment of periodontitis [1]. However, relationship between the yield of singlet oxygen and the bactericidal effect has not been fully understood. Although the yield of singlet oxygen is determined by indirect analytical methods such as electron spin resonance (ESR) technique [2], analysis of 1,3-diphenylisobenzofuran (DPIBF) oxidation [3], and application of fluorescent probes [4], the determinations of photogenerated singlet oxygen have not been fully elucidated so far. Therefore, the aim of this review article is to discuss the appropriate analytical methods for photogenerated singlet oxygen from the aspect of drawback and advantage.

2 The Principle of Each Analytical Method

The ESR technique cannot determine directly singlet oxygen because singlet oxygen is not free radical. However, nitroxide radical generated by a reaction between singlet oxygen and sterically hindered amine can be measured by ESR, leading to an indirect analysis of singlet oxygen. In a spectrophotometric analysis of DPIBF, the absorption peak of DPIBF at 420 nm is measured, since the absorption peak decreases in proportion to the amount of singlet oxygen. Several fluorescent probes have been also developed and used for detection of singlet oxygen. The fluorescent probe is designed to be photoexcited when it reacts with singlet oxygen.

3 Factors Affecting Each Analytical Method

Determination of singlet oxygen can be affected by spectrophotometric properties of the methods and the characteristic features of photosensitizers. Therefore, factors affecting each analytical method were discussed in reference to the evaluation using rose bengal as a representative photosensitizer. The sensitivity of the analytical methods was in the order of DPIBF < ESR < SOSG. However, SOSG could be used only when the concentration of rose bengal was very low (< 1 μ M) because fluorescence from SOSG is interfered by the rose bengal itself. In addition, since the absorption spectrum of DPIBF was considerably changed by the laser irradiation at 405 nm, photosensitizers which are excited by light with a wavelength of around 400 nm such as hematoporphyrin cannot be used in the DPIBF oxidation method. On the other hand, the ESR analysis using a sterically hindered amine, especially 2,2,6,6-tetramethyl-4-piperidinol and 2,2,5,5-tetramethyl-3-pyrroline-3-carboxamide, had proper sensitivity and wide detectable range for the yield of photogenerated singlet oxygen.

4 Conclusion

It is suggested that the yield of singlet oxygen generated in PDT using various photosensitizers can be evaluated properly by the ESR analysis.

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Time-Specific and Stage-Specific Expression of *TP63* Gene During Osteoblast Differentiation

Viviane K.S. Kawata, Takashi Matsuura, Masanobu Satake,
and Shuntaro Ikawa

Abstract. p63 is a member of a highly conserved p53 transcription factor family, which regulates complex biological processes during development and tumorigenesis. Although extensive analyses *TP63* gene using cultured cells of epidermal lineage led to acquisition of ample knowledge regarding its regulatory roles in epidermal differentiation, *TP63* function in normal development of osteoblasts remains largely unknown. Thus, we examined expression levels of individual p63 isoforms during the course of MC3T3-E1 osteoblast differentiation using Western blot and RT-PCR assays. TAp63 γ expression was induced at the early phase of differentiation and continued throughout this process. TAp63 α expression was induced

Viviane K.S. Kawata

Department of Molecular Immunology, Tohoku University Graduate
School of Medicine, Sendai 980-8575, Japan
and

Division of Periodontics and Endodontics, Tohoku University Graduate
School of Dentistry, Sendai 980-8575, Japan
and

Ikawa Group, Center for Interdisciplinary Research, Tohoku University,
Aoba-ku, Sendai 980-8578, Japan

T. Matsuura

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate
School of Dentistry, Sendai 980-8575, Japan
and

Ikawa Group, Center for Interdisciplinary Research, Tohoku University,
Aoba-ku, Sendai 980-8578, Japan

M. Satake

Department of Molecular Immunology, Tohoku University Graduate
School of Medicine, Sendai 980-8575, Japan

S. Ikawa (✉)

Ikawa Group, Center for Interdisciplinary Research, Tohoku University,
Aoba-ku, Sendai 980-8578, Japan
e-mail: sikawa@cir.tohoku.ac.jp

at the late phase of differentiation paralleling with that of osteocalcin. In addition, despite the up regulation of Δ Np63 transcripts was also detected by RT-PCR, Δ Np63 isoform was undetectable by Western blot experiment. These results suggest that TAp63 isoforms may regulate osteoblast differentiation.

Key words. Differentiation, Osteoblastic markers, Osteoblasts, p63, Proliferation

1 Introduction

Tumor suppressor p63 (also known as p51 [1]) is a homologue of p53 and p73, consist a family of highly conserved p53 transcription factors, which plays essential roles in development and tumorigenesis. Mainly through its transactivation function, p63 regulates complex biological processes. Despite p63 function is extensively studied in epidermal cells [2–4], little is known regarding the molecular basis of p63 isoforms in non-epidermal lineage. Thus, biological significance of p63 obtained in our study will be presented.

2 Results

Regulated expression of each of p63 isoforms during osteogenic differentiation of MC3T3-E1 cells.

To evaluate the role of p63, we examined its expression in murine osteoblastic cell line MC3T3-E1 during differentiation in vitro. MC3T3-E1 cells undergoes osteogenic differentiation when cells are contact inhibited. When these cells were analyzed for expression of Osteocalcin (*OCN*), osteogenic differentiation marker, high levels of its expression was observed at 13 and 21 days of culture period, indicating that the cells underwent osteogenic differentiation before day 13. We then examined the expression of each of p63 isoform during osteogenic differentiation. Small amount of TAp63 γ was detected in undifferentiated proliferative phase (day 0). Interestingly, this phase was followed by an immediate induction of TAp63 γ and its expression further increased as the differentiation progressed (6, 13 and 21 days). In contrast, TAp63 α was not detected in an undifferentiated proliferative phase (day 0). Notably, however, this isoform was rapidly induced in day 6 at the onset of osteogenic differentiation. In addition to Western blot experiments, we also conducted RT-PCR analyses detecting each of TAp63 and Δ Np63 isoforms. This experiment largely confirmed the result obtained by the Western blot analyses. However, although Δ Np63 protein was not detected in the Western blot analyses, its transcript was clearly detected by RT-PCR analyses. We first hypothesized that this discrepancy was due to vigorous degradation of Δ Np63. However, Δ Np63 isoforms remained undetectable even in the presence of the proteasomal inhibitor, which led to dramatic stabilization of TAp63 α and TAp63 γ isoforms. We speculate that the translation of Δ Np63 mRNA is inhibited by miRNA or other mechanisms.

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Metal–Allergy Cross-Reactions in Mice

Masayuki Kinbara, Yasuhiro Nagai, Teruko Takano-Yamamoto,
Shunji Sugawara, and Yasuo Endo

Abstract. Few adequate animal models exist for metal-allergies. However, we previously found that lipopolysaccharide (LPS) promote metal-allergies in mice, and we thereby established a reproducible murine metal-allergy model. Information concerning cross-reactivity among metals is still insufficient, especially lacking the studies in vivo. Here, we used this murine model to examine allergic cross-reactions among some metals. In the experiments using ultra-pure metals (Ni, Pd, Co, Cr, and Cu), Ni and Pd cross-reacted, and their minimum allergy-inducing concentrations were similar. However, there were no cross-reactions among other tested metals. Surprisingly, in the experiments using low-purity metals, all the tested metals (Ni, Pd, Co, Cr, and Cu) cross-reacted. Our findings suggest that there is cross-reactivity between Ni and Pd in the tested metal (Ni, Pd, Co, Cr, and Cu) and high-purity metal salts should be considered for use in clinical patch-testing.

Key words. Cross-reaction, Hypersensitivity, Inflammation, Lipopolysaccharide, Metal-allergy

M. Kinbara (✉)

Department of Orthodontics and Dentofacial Orthopedics, Graduate School of Dentistry,
Tohoku University, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
and

Department of Molecular Regulation, Graduate School of Dentistry, Tohoku University,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: kinbara@dent.tohoku.ac.jp

Y. Nagai, S. Sugawara, and Y. Endo

Department of Molecular Regulation, Graduate School of Dentistry, Tohoku University,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

T. Takano-Yamamoto

Department of Orthodontics and Dentofacial Orthopedics, Graduate School of Dentistry,
Tohoku University, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

Contact allergy to metals is the most common form of allergic contact dermatitis. It remains to be determined the detailed mechanism of metal-allergies, including metal-binding proteins. Few adequate animal models exist for metal-allergies, it being especially difficult to induce metal-allergies in mice. However, we previously reported that lipopolysaccharide (LPS) promoted metal-allergies in mice at both sensitization and elicitation steps, and we thereby established a reproducible murine metal-allergy model termed 'Metal(+LPS)-allergy' (e.g., Ni(+LPS)-allergy, etc.) [1, 2]. Many patients with metal-allergy exhibit positive responses to several metals such as Ni, Pd, and Co. Because information concerning cross-reactions among metals is still insufficient, especially lacking the studies *in vivo*, it is difficult to judge whether such plural reactions reflect the sensitizations to individual metals or cross-reactions. Here, we used our previously described murine metal-allergy model to examine allergic cross-reactions among some metals. In experiments using ultra-pure metal salts (Ni, Pd, Co, Cr, and Cu), Ni and Pd cross-reacted (i.e., Ni-sensitized mice displayed ear-swelling upon challenge with Pd, and vice versa). However, there were no cross-reactions among other tested metals. In Ni(+LPS)-sensitized mice, the minimum allergy-inducing concentrations (MAICs) of Ni (true antigen) and Pd (cross-react antigen) were in the same level. However, the combination of Ni and Pd at their sub-MAICs additively induced their allergic reactions. Surprisingly, low-purity Ni(+LPS)-sensitized mice reacted to all the tested low-purity metals (Ni, Pd, Co, and Cr). The data suggest that (1) among Ni, Pd, Co, Cr, and Cu, there is cross-reactivity between Ni and Pd (members of the same group in the periodic table of elements), but not among other metals, (2) Ni and Pd may display an identical or very similar spatial geometry in their protein-bound state, and thus cross-reactions might be mediated by the same mechanism, and (3) high-purity metal salts must be used for identifying metal-allergens by patch-testing.

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The Stomach's Response to Sham Feeding: Cephalic Vagal Effects on Postprandial Gastric Motility

Yuko Komine and Yoshinori Hattori

Abstract. The aim of this study was to clarify the effects of sham feeding followed by feeding of food on postprandial gastric motility by using EGG. Postprandial EGG was monitored for 60 min. Each subject consumed the meal three times under fine chewing (FC), coarse chewing (CC), and coarse chewing preceded by gum chewing (CC+G) condition. The number of the time points when gastric motion was active was compared among the three conditions. In the middle and the last 20 min, the number was significantly greater in FC than in CC. Sham feeding before meal may help to enhance postprandial gastric motility.

Key words. Electrogastrography, Gastric motility, Mastication, Sham feeding

1 Introduction

Sham feeding as well as feeding encourages gastrointestinal motility through cephalic-vagal stimulation [1, 2]. However, their interaction is still unclear. This study aimed to investigate the effects of sham feeding followed by feeding of food on gastric motility by cutaneous electrogastrography (EGG).

Y. Komine (✉) and Y. Hattori

Division of Aging and Geriatric Dentistry, Department of Oral Function and Morphology,
Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai, Japan
e-mail: y_komine@dent.tohoku.ac.jp

2 Materials and Methods

Postprandial EGG was monitored for 60 min after meal ingestion (396 kcal) in nine healthy male adults after 30 min of baseline recording. Each subject consumed the meal three times under different chewing conditions as follows: (1) fine chewing (FC; subjects chewed the food thoroughly), (2) coarse chewing (CC; subjects swallowed the food with minimum chewing), and (3) coarse chewing preceded by gum chewing for 10 min as a form of sham feeding (CC+G). The 3-cpm EGG power was calculated every 2.5 min. Gastric motion was defined to be active in case the power exceeded the threshold determined from its baseline value. The number of the time points when gastric motion was active was compared among the three conditions by using the Chi-square test.

3 Results and Discussion

The total number of time points was 115, 63, and 101 in FC, CC, and CC+G conditions, respectively. In the first 20 min after meal, no significant difference was found in the number of active time points among the three conditions. In the middle and the last 20 min, the number of active time points was significantly greater in fine chewing than in coarse chewing. The value in coarse chewing with gum chewing condition was in between them (Fig. 1). These results demonstrate that the increase of masticatory activity caused by sham feeding encourages postprandial bowel motility.

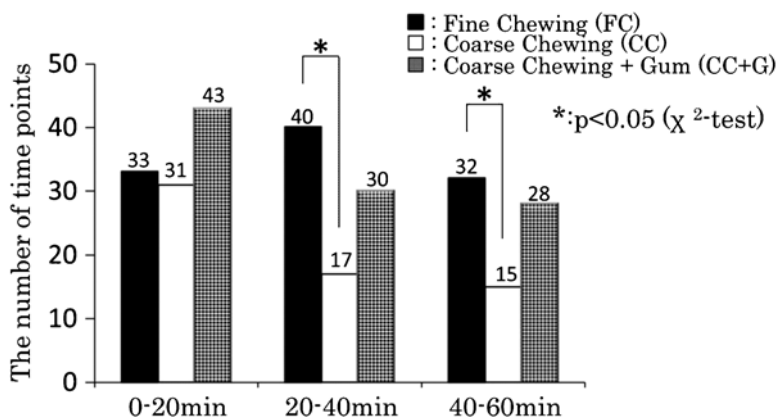


Fig. 1. During the second and third 20-min, the number was significantly lower in CC (white areas) than in FC (black areas) conditions. The difference disappeared by adding gum chewing prior to food ingestion. The active time points of CC+G (shaded areas) during these periods were almost double that of CC

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Mechanical Properties and Degradability of HA/PLLA Composites with Different Particle Size Distribution

Tetsuo Takayama, Mitsugu Todo, and Hiroshi Ito

Abstract. Reduction behavior of bending properties of HA/PLLA composites with different particle size distribution were investigated using a phosphate buffer solution. The reduction behavior of bending strength and bending modulus depended on the particle size distribution, suggesting that degradability can be controlled by controlling particle size distribution.

Key words. Composites, Hydrolysis degradation, Mechanical properties, Particle size distribution

Hydroxyapatite (HA)/poly(L-lactic acid) (PLLA) composite materials have higher rigidity and biocompatibility compared to neat PLLA, and also have excellent bone conduction capacity. These materials have therefore been used as bone fixation devices, and also considered to be used as another device such as scaffold in bone regenerative medicine. In order to enlarge the application range, the material needs to be multi-functional and highly functional of material essentially. It is well known that the mechanical properties of particle dispersed composites depend on the interfacial structure, dispersibility and the particle size. However, it was not reported about the effects of distribution of particle size. In this study, therefore, the effects of particle size distribution on the mechanical properties such as bending strength and modulus were investigated.

Two kinds of HA particles (Sangi Co. Ltd) with different shapes were used as filler. They are called as micro-HA (5 μm) and nano-HA (100 nm) on the basis of

T. Takayama (✉) and H. Ito
Graduate School of Science and Engineering, Yamagata University,
Yonezawa, Yamagata 992-8510, Japan
e-mail: t-taka@yz.yamagata-u.ac.jp

M. Todo
Research Institute for Applied Mechanics, Kyushu University,
6-1 Kasuga-koen, Kasuga, Fukuoka 816-8580, Japan

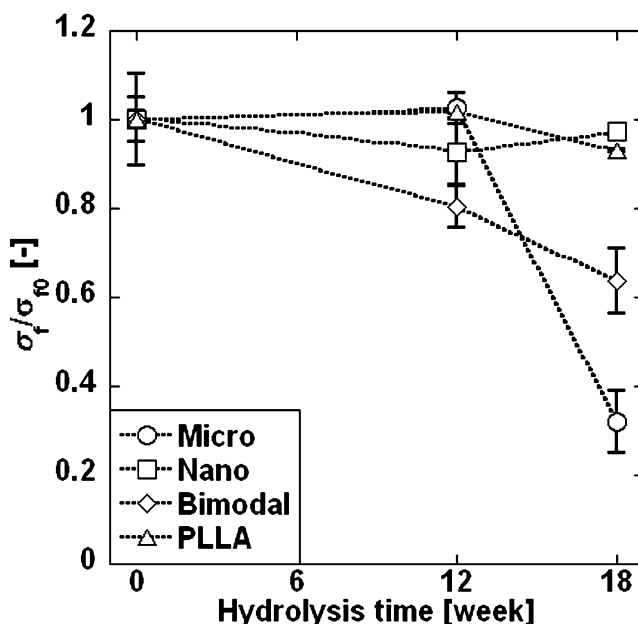


Fig. 1 Degradation of PLLA and HA/PLLA in PBS at 37°C

their shapes, where the numbers in parentheses are their representative sizes. PLLA was used as matrix. HA/PLLA mixtures were prepared from the PLLA pellets and the HA particles using a conventional melt-mixer by mixing for 20 min at a rotor speed of about 50 rpm at 190°C. HA plates of 140×140×2 mm² were fabricated from these mixture by re-melting at 190°C and pressed at 30 MPa for 30 min using a hot press. The plates were then quenched to room temperature using a water cooling system. Beam specimens were made from the HA/PLLA plates for hydrolysis degradation. Hydrolysis degradation under 37°C was conducted by using a phosphate buffer solution (PBS) with pH = 7.4. Hydrolysis time was 12 and 18 weeks. After hydrolysis, specimens were dried in a conventional oven at 37°C for more than 24 h. Three-point bending tests were then conducted at a loading-rate of 10 mm/min by a servo-hydraulic testing machine. Bending strength and bending modulus were evaluated by complying with JIS K7171. In this study, reduction behavior of bending strength and modulus was evaluated as degradability.

Figure 1 shows the degradation behavior of PLLA and HA/PLLA in PBS at 37°C. It was found that the reduction behavior of strength and modulus depended on the particle size distribution, suggesting that degradability can be controlled by controlling particle size distribution.

Expression of Ten-m/Odz3 in the Fibrous Layer of Mandibular Condylar Cartilage and the Early Stage of Chondrogenic Differentiation of ATDC5 Cells

Nobuo Takeshita, Takashi Murakami, Tomohiro Fukunaga,
Koichi Hiratsuka, Yoshimitsu Abiko, Takashi Yamashiro,
and Teruko Takano-Yamamoto

Abstract. Temporomandibular joint is essential for normal development and function of jaw. Mandibular condylar cartilage histologically consists of fibrous and cartilage layers. It has been speculated that fibroblast-like cells in the fibrous layer differentiate into chondrocytes in the process of condylar development. However, molecular mechanisms regulating the process have not been cleared. We revealed that Ten-m/Odz3 was highly expressed in the fibrous layer compared with the cartilage layer. Ten-m/Odz3 was strongly expressed in fibrous and proliferating chondrocyte layers in mandibular condylar cartilage and in perichondrium and proliferating chondrocyte layer in femoral cartilage during the early stage of development. Furthermore, Ten-m/Odz3 was expressed during the early stage of the chondrogenic differentiation of ATDC5 cells. These findings suggest that Ten-m/Odz3 acts as a regulator during the early development of cartilage and may modulate the differentiation of fibroblast-like cells into chondrocytes in the mandibular condyle.

Key words. ATDC5, Mandibular condylar cartilage, Ten-m/Odz3

Temporomandibular joint is essential for normal development and function of jaw. Mandibular condylar cartilage histologically consists of fibrous and cartilage layers during the development. It has been speculated that fibroblast-like cells in the fibrous

N. Takeshita, T. Fukunaga, and T. Takano-Yamamoto (✉)
Division of Orthodontics and Dentofacial Orthopedics, Tohoku University
Graduate School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: t-yamamo@m.tohoku.ac.jp

T. Murakami and T. Yamashiro
Department of Orthodontics, Okayama University Graduate School of Medicine,
Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8525, Japan

K. Hiratsuka and Y. Abiko
Department of Biochemistry and Molecular Biology, Nihon University School
of Dentistry at Matsudo, 2-870-1 Sakae-cho Nishi, Matsudo 271-0061, Japan

layer may have a role as a source of undifferentiated mesenchymal cells that subsequently differentiate into chondrocytes [1, 2]. Ten-m/Odz is a type II transmembrane protein in the form of a homodimer and is involved with cell–cell adhesion, cytoskeleton, and signal transduction [3]. It has been reported that Ten-m/Odz is expressed during neuronal tissue development. Ten-m/Odz is also expressed in non-neuronal tissues, such as the pharyngeal arches, some somites, and limb buds. However, the expression of Ten-m/Odz in mandibular condylar cartilage has not been investigated. In the present study, we examined expression pattern of Ten-m/Odz3 in mandibular condylar and femoral cartilage in growing postnatal mice, as well as ATDC5 cells, a mouse chondrogenic cell line.

To compare the expression level of Ten-m/Odz3 between in fibrous and cartilage layers, we digested each area from criostat sections by laser-capture microdissection, and then analyzed gene expression by RT-PCR. As a result, the expression level of Ten-m/Odz3 in fibrous layer was higher than that in cartilage layer. Then we performed in situ hybridization to assess the temporal and spatial expression of Ten-m/Odz3 in mandibular condylar cartilage. Ten-m/Odz3 was expressed in the fibrous and proliferating chondrocyte layers, which expressed type I collagen. Weak Ten-m/Odz3 expression was detected in the mature chondrocyte layer. In femoral cartilage, Ten-m/Odz3 mRNA was strongly expressed in perichondrium and proliferating chondrocyte layer. Furthermore, Ten-m/Odz3 was strongly expressed in the early stage of chondrogenic differentiation of ATDC5 cells, and the expression pattern was similar to that of type I collagen.

In summary, Ten-m/Odz3 was expressed in the early stage of chondrogenic differentiation in vivo and in vitro. The function of Ten-m/Odz3 during cartilage development still remains unclear. However, it is conceivable that Ten-m/Odz3 acts, at least in part, as a regulator of the cartilage development and may modulate the differentiation of fibroblast-like cells into chondrocytes in the mandibular condyle.

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Roles of IL-6 in Mastication in Mice and Effects of Training and Food Hardness

Masahiro Tsuchiya, Tomomi Kiyama, Shinobu Tsuchiya,
Hirohisa Takano, Eiji Nemoto, Keiichi Sasaki,
Shunji Sugawara, Yasuo Endo, and Makoto Watanabe

Abstract. Upon exercise, IL-6 produced by skeletal muscles plays a key role in glucose homeostasis in skeletal muscles during physical exercise. In this article, the role of IL-6 in masticatory activity (MA) were reviewed, especially in the unique model of gnawing behavior (GB) in mice. GB induced IL-6 mRNA expression in masseter muscles and also increased SOCS-3 mRNA expression, a downstream molecule of IL-6 signaling. Interestingly, mice fed with harder pellet food exhibited higher MA and also lower IL-6 mRNA expression in masseter muscle. IL-6-deficient mice exhibited much lower MA. In addition, in masseter muscle, the recovery

M. Tsuchiya (✉)

Division of Aging and Geriatric Dentistry, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: tsuchiya-thk@umin.ac.jp; tsuchiya@mandible.dent.tohoku.ac.jp

T. Kiyama and K. Sasaki

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

S. Tsuchiya

Division of Oral Dysfunction Science, Tohoku University Graduate School
of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

H. Takano

Environmental Health Sciences Division, National Institute
for Environmental Studies, 16-2 Onogawa, Tsukuba 305-8506, Japan

E. Nemoto

Division of Periodontology and Endodontology, Tohoku University Graduate
School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

S. Sugawara and Y. Endo

Division of Oral Immunology, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

M. Watanabe

Department of Health Services Management, Tohoku Fukushi University, Kansei
Fukushi Research Center, 6-149-1, Kunimigaoka, Aoba-ku, Sendai 989-3201, Japan

of glycogen level after GB and the Glut4 mRNA expressions were lower in IL-6-deficient mice.

Taken together, IL-6 produced in masseter muscle in response to mastication plays a key role in sustaining normal masticatory activity by supporting glucose homeostasis, and that daily provision of hard food increases MA, with a decreasing dependency on IL-6 production.

Key words. Fatigue, IL-6, Mastication, Skeletal muscle

1 IL-6 During Muscle Activity

Recently, interleukin (IL)-6, a major proinflammatory cytokine, is rapidly released into the circulation during skeletal muscle contraction, and acts in a hormone-like fashion to increase systemic glucose uptake and fat oxidation [1]. Hence, IL-6 is now believed to be an essential regulator in the maintenance of muscle activity during exercise. Masseter muscles are typical slow-twitch dominant muscles, interestingly, which reportedly contain more IL-6 than fast-twitch muscles. Previously a unique model for quantitatively analyzing masticatory activity (MA) in mice was established [2]. Using this unique model for measuring gnawing behavior (GB), IL-6 production during GB and relationship between IL-6 and MA were examined.

2 A Model Gnawing Behavior in Mice and Analyzing of IL-6

The details of methods were reported previously [2]. Briefly, male 8-weeks-old mice were kept under the standard condition before use. When a mouse is restrained in a cylinder blocked with a plastic strip, it spontaneously gnaws the strip away to escape, being termed as gnawing behavior (GB). Hence, the weight reduction of strip can be used as an index of MA. The mRNA expression level of IL-6, SOCS-3, a downstream gene of IL-6 signaling and glucose transporter-4 (Glut-4) were analyzed by quantitative RT-PCR. Statistical analysis was performed by Student's *t*-test or by one-way ANOVA followed by the Bonferroni procedure for multiple comparisons ($P < 0.05$).

3 IL-6 and SOCS-3 mRNA Expression in Masseter Muscles

IL-6 mRNA expression in masseter muscle was dramatically increased with prolonged GB, while the glycogen content in the masseter muscle decreased during GB. In addition, SOCS3 mRNA expression in masseter muscle increased significantly. These suggest that induction of IL-6 mRNA is involved in the muscle fatigue caused by GB.

4 MA of IL-6-KO

MA of IL-6-KO mice decreased faster than that of WT mice. In IL-6-KO mice, the glycogen level in masseter muscle after GB was significantly lower than that in wild type mice, and there was no GB-induced expression of Glut4 mRNA expression, suggesting that glycogen recovery is insufficient in IL-6-KO mice. Namely, IL-6 plays a significant role in maintaining glucose homeostasis in the masseter muscle during GB.

5 Effect of Hardness of Daily Food on MA and on Induction of IL-6 mRNA

To evaluate the effect of animals' daily food hardness on MA and IL-6 expression, either pellet or powdered food was given to mice for 3 weeks from their weaning. MA in the pellet-raised group was markedly higher than that in the powder-raised group, and there was no GB-induced IL-6 mRNA expression in the pellet-raised group.

6 Conclusions

When masticatory activity is unusually high, IL-6 produced in the masseter muscle plays an essential role to sustain the masticatory activity by retaining the level of glucose.

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SDF-1 Regulation of Expression on Periodontal Ligament Cells Derived from Human Permanent Teeth

Takeyoshi Asakawa, Naoyuki Chosa, Tomokazu Hasegawa,
Asami Asakawa, Akira Isizaki, and Mituro Tanaka

Abstract. Both periodontal ligament tissue that located between the alveolar bone and the root of teeth and dental pulp tissue are reported to have mesenchymal stem cells [1, 2] (MSCs). MSCs are considered to be involved in the homeostasis of periodontal tissue. Recent studies have revealed that the stromal cell-derived factor-1 α (SDF-1) is crucial for migration of endothelial precursor cells and MSCs through its receptor, CXCR4. However, regulation of SDF-1 expression in the periodontal ligament cells is still unknown. In this study, we investigated whether FGF-2 could regulate the expression of SDF-1 in periodontal ligament cells derived from human permanent teeth.

Key words. Fibroblast growth factor-2, Periodontal ligament cells, Permanent tooth, Stromal cell-derived factor 1

1 Isolation of Periodontal Ligament Cells

The periodontal ligament cells (PDLs) were isolated from extracted human teeth for treatment. PDLs cultured in α -MEM supplemented with 10% FBS in the absence or presence of 10 ng/ml FGF-2. The cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂ in air.

T. Asakawa, A. Asakawa, and M. Tanaka
Department of Pediatric Dentistry, School of Dentistry, Iwate Medical University,
19-1 Uchimarui, Morioka 020-8505, Japan

N. Chosa and A. Isizaki
Department of Oral Biochemistry, School of Dentistry, Iwate Medical University,
19-1 Uchimarui, Morioka 020-8505, Japan

T. Hasegawa (✉)
Department of Pediatric Dentistry, Tokushima University Hospital,
3-18-15 Kuramoto, Tokushima 770-8504, Japan
e-mail: hasegawa@dent.tokushima-u.ac.jp

2 Expression of SDF-1

Total RNA was extracted from the cultured PDLC. A Thermal Cycler Dice Real-time system (Takara Shuzo) was used for the two-step reverse transcription-polymerase chain reaction. Specific oligonucleotide primer for target sequences encoding parts of SDF-1. After treatment with FGF-2 for 7 days, the conditioned media from PDL cell culture were collected for SDF-1 samples. Then, 20 μ l of conditioned media were dissolved in SDS buffer without dithiothreitol, incubated at 95°C for 5 min, and resolved electrophoretically on 10% SDS-polyacrylamide gels and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA). After being blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBST), the membrane was incubated with mouse anti-human SDF-1 antibodies and subsequently with anti-mouse secondary antibodies (ZYMED laboratories Inc., San Francisco, CA). Specific protein bands on the membrane were detected by using an enhanced AP conjugate substrate kit (BIO RAD laboratories, CA) as described previously.

3 Results and Discussion

SDF-1 mRNA expression was significantly decreased by the treatment with FGF-2, it was recovered when PDLCs were treated with both FGF-2 and its receptor inhibitor, SU5402 (Fig. 1). The production of SDF-1 expression by Western blot analysis became the similar result. It was considered that FGF-2 could regulate SDF-1 expression in PDLCs.

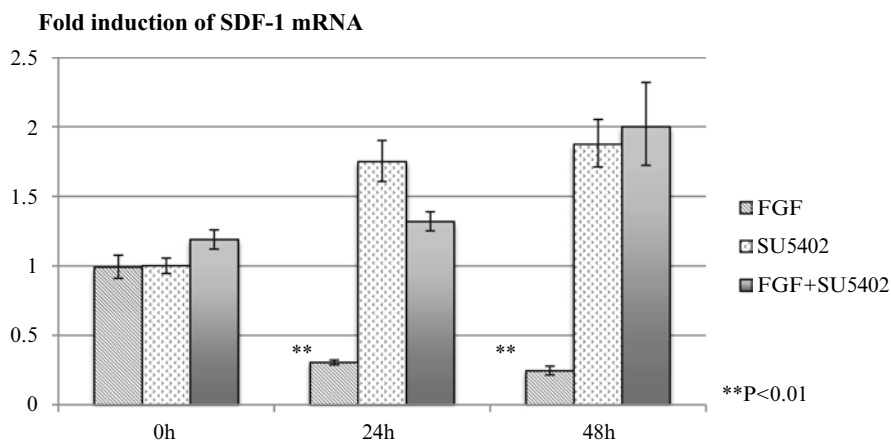


Fig. 1. PDLCs cultured in α -MEM supplemented with 10% FBS in the absence of 10 ng/ml FGF-2 (FGF). In addition PDLCs were treated with FGF-2 receptor inhibitor (SU5402) or both FGF-2 and SU5402 (FGF+SU5402) for 0 hour (0 h), 24 hours (24 h) or 48 hours (48 h)

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Micro-spatial Environment and Osteoblast Osteogenesis

Mirei Chiba, Ryosuke Miyai, and Haruhide Hayashi

Abstract. Various biomaterials that provide a structural scaffold and a micro-spatial environment have been developed for bone regeneration. However, the effects of the size of the micro-space available for osteoblasts are not known. Using a photolithographic technique, the effects of the micro-spatial environment on osteoblast proliferation and differentiation were investigated in cultures plated on modified patterned surfaces. The results suggest that optimal space for osteoblasts is important for osteogenesis. Furthermore, molecules that recognize the extracellular environment related to cell adhesion and movement, might be involved in micro-space-induced osteoblast osteogenesis.

Key words. Bone regeneration, Micro-space, Osteoblasts, Scaffold

1 Introduction

Recent advances in cell biology, dentistry, and tissue engineering have led to the development of new therapeutic agents and novel materials for the repair of large bone defects caused by trauma, congenital defects, or bone tumors. These bone repair strategies utilize degradable three-dimensional structural scaffolding. Material characteristics such as hydrophobicity, crystallinity, and degradability affect the cell activity involved in bone formation. Furthermore, osteoblasts, osteoclasts, and their progenitor cells have been investigated in determining the issues that influence effective scaffold design for bone repair.

M. Chiba (✉), R. Miyai, and H. Hayashi
Division of Oral Physiology, Department of Oral Function and Morphology,
Graduate School of Dentistry, Tohoku University, 4-1 Seiryō-machi, Aoba-ku,
Sendai 980-8575, Japan
e-mail: mirei@m.tohoku.ac.jp

2 Bone Regeneration and Scaffold for Tissue Regeneration

At least three elements are necessary for tissue regeneration: a cell source for incorporating into regenerating tissues and organs, a growth factor for the growth and development of cells, and a scaffold to guide the process. The scaffold acts as a cell-adhesion substrate, providing space for regenerative tissue and preventing invasions from neighboring tissue (e.g., scar tissue). It can be molded into the form of the target tissue that is being repaired and it is porous, providing a way for oxygen and nutrition to reach the cells for growth factor regulation, retention and release. However, little is known regarding how the size of a scaffold affects cell proliferation and differentiation and, in particular, whether the amount of micro-space available affects these processes.

One of the most important influences on osteoblast reactivity is the three-dimensional morphology of the substrate, which includes the size, shape, and surface texture of the material. However, while it is known that bone cells are sensitive to the morphology of a material, little is known about the mechanism.

3 Micro-spatial Environment

Many studies have demonstrated that cell shape and orientation are related to gene expression. Additionally, cells may change shape after adhering to a material surface, leading to different cell phenotypes and cytoskeletal changes that may affect cellular metabolism. Therefore, the size and shape of the particles used in biomaterials have been investigated because they are recognized as affecting osteoblast proliferation and differentiation.

Although, the characteristics of the biomaterials used in osteoblast scaffolds have been investigated, the effects of the amount of micro-space available have not. Therefore, we investigated the effects of the micro-spatial environment on osteoblast proliferation and differentiation using a photolithographic technique. Osteoblastic cells were cultured in culture plates in which the bottom was modified with a micro-box patterned surface.

4 Discussions and Conclusions

The amount of micro-space available to osteoblasts affected their proliferation and differentiation, demonstrating the importance of having optimal space for osteogenesis. The molecules involved in extracellular environment recognition (i.e., those related to cell adhesion and movement) might participate in micro-space-induced osteogenesis.

Acknowledgements The authors acknowledge a Grant-in-Aid for Scientific Research (C) 22592278 (to M.C.) from Japanese Ministry of Education, Science, Sports, and Culture.

Gene Expression in Human Osteoblasts and Periodontal Ligament Cells Under Compressive Force

Mirei Chiba, Ryosuke Miyai, and Haruhide Hayashi

Abstract. During orthodontic tooth movement, various types of mechanical stresses are loaded on the periodontal tissue. In response to these stresses, remodeling of periodontal tissue occurs. The periodontal ligament is considered to have an important role in triggering a series of orthodontic force-induced tissue reactions. Using global gene expression analysis, we investigated the similarities and differences in cell-type-specific gene expression patterns induced by compressive force on osteoblasts and periodontal ligament cells.

Key words. Compression force, Gene expression, Osteoblasts, Periodontal ligament cells, Remodeling

1 Introduction

The application of orthodontic force induces tooth movement. During this mechanical stress, cells in the periodontal ligament directly receive the mechanical force, and remodeling of the periodontal tissue occurs. The periodontal ligament is important for remodeling the alveolar bone and periodontal tissue. Clinically, an ankylosed tooth lacking a periodontal ligament does not move even when orthodontic force is applied. Similarly, dental implants lack periodontal ligament, and do not move even if an orthodontic force is applied. In these cases, the immobile tooth is

M. Chiba (✉), R. Miyai, and H. Hayashi
Division of Oral Physiology, Department of Oral Function and Morphology,
Graduate School of Dentistry, Tohoku University, 4-1 Seiryō-machi, Aoba-ku,
Sendai, 980-8575, Japan
e-mail: mirei@m.tohoku.ac.jp

used as an anchor for tooth movement during mechanical orthodontic treatment. The periodontal ligament is considered to play an important role in triggering a series of orthodontic force-induced tissue reactions. As tissue response first occurs at the compression site, the initial compression is an important signal for tissue response. However, the mechanism of compressive-force-induced periodontal tissue remodeling is not clear.

2 Global Gene Expression Analyses

In a previous study, we demonstrated that both osteoblasts and periodontal ligament cells were affected by a compressive force applied during periodontium remodeling. To understand the homeostasis of periodontal tissue and to elucidate the mechanisms when it reacts to or resists mechanical stress, it is useful to analyze the cell-type-specific effects of compressive force on gene expression in human osteoblasts and periodontal ligament cells by gene profiling using microarray analysis. In this study, we investigated the similarities and differences of the expression patterns detected by global gene expression analysis. This expression pattern analysis suggested that both properties common among cells and cell-specific properties affect the remodeling mechanism of periodontal tissues in response to compressive force.

3 Conclusions

Identification of a cell-type-specific gene expression pattern in osteoblasts and periodontal ligament cells in response to compressive force is important for understanding orthodontic tooth movement. Our data provide a unique and comprehensive global picture of gene activity related to compressive force. Some of the expressed genes probably contribute to periodontal remodeling.

Acknowledgement The authors acknowledge a Grant-in-Aid for Scientific Research (C) 22592278 (to M.C.) from Japanese Ministry of Education, Science, Sports, and Culture.

Establishment of Clonal Periodontal Ligament Cell Line Derived from Deciduous Tooth Immortalized by Human Telomerase Reverse Transcriptase (*hTERT*) Gene Transfer

Tomokazu Hasegawa, Naoyuki Chosa, Takeyoshi Asakawa,
Yoshitaka Yoshimura, Akira Ishisaki, and Mitsuro Tanaka

Abstract. Recently, many studies have shown that mesenchymal stem cells (MSCs) exist in dental pulp and periodontal ligament (PDL) of human deciduous teeth. MSCs derived from extracted teeth have been considered an appropriate candidate for not only dental tissue regeneration but also for the treatment of general diseases. However, there was no immortal PDL cell line derived from human deciduous teeth for cell-based regeneration study. In this study, we established tree PDL cell lines derived from human deciduous tooth immortalized by the transfection with human telomerase reverse transcriptase (*hTERT*) gene. The transfected cells were named SH, Single cell from Human periodontal ligament. All SH clones expressed *hTERT* and scleraxis, periostin, cementum-derived protein 23 and tenomodulin. Though all SH clones expressed osteoblastic marker genes, only the clone, SH 9 cells, differentiated mature osteoblasts. These clones could be useful for the research in regenerative medicine.

Key words. Deciduous tooth, *hTERT*, Immortalize, Periodontal ligament cells

T. Hasegawa (✉)

Department of Pediatric Dentistry, School of Dentistry, Iwate Medical University,
19-1 Uchimar, Morioka 020-8505, Japan

and

Department of Pediatric Dentistry, Tokushima University Hospital,
3-18-15 Kuramoto, Tokushima 770-8504, Japan

e-mail: hasegawa@dent.tokushima-u.ac.jp; hasegawa@iwate-med.ac.jp

N. Chosa and A. Ishisaki

Department of Oral Biochemistry, School of Dentistry, Iwate Medical University,
19-1 Uchimar, Morioka 020-8505, Japan

T. Asakawa and M. Tanaka

Department of Pediatric Dentistry, School of Dentistry, Iwate Medical University,
19-1 Uchimar, Morioka 020-8505, Japan

Y. Yoshimura

Division of Oral Pathological Science, Department of Molecular Cell Pharmacology,
Hokkaido University Graduate School of Dental Medicine,
Kita 13 Nishi 7, Kita-ku, Sapporo 060-8586, Japan

1 Introduction

Periodontal ligament (PDL) is a fibrous connective tissue located between the tooth root and alveolar bone. PDL tissue consists of heterogeneous cell population such as fibroblasts, cementoblasts, osteoblasts, endothelial cells, osteoclasts and certain types of nerve cells. Recently, it has been showed that PDL cells derived from human teeth have the properties similar to the mesenchymal stem cell (MSC) in bone marrow [1, 2]. Autogenous MSCs transplantation is thought to be one of the most promising therapeutic concepts because it may solve many problems including immunogenicity of allogenic grafts. MSCs isolated from deciduous teeth could be one of ideal sources since it is easy, inexpensive, safe, and less-invasive procedure to extract deciduous teeth compared to bone marrow aspiration. Therefore, an immortalized PDL cell line derived from deciduous teeth could be a useful tool for the research of regenerating medicine and investigating stem cell-based therapy. In this study, we established immortalized PDL cells derived from deciduous teeth and showed osteogenic properties.

2 Materials and Methods

Human PDL cells were isolated from clinically healthy extracted deciduous teeth. The ethics committee of Iwate Medical University, School of Dentistry approved our experimental protocols (No.01101). Cells were placed on culture dishes and maintained in alpha-modified minimum essential medium (α -MEM; GIBCO BRL, Gaithersburg, MD, USA) supplemented with 10% foetal bovine serum (FBS; GIBCO BRL). Fibroblastic cells that grew from the PDL tissue culture were used as PDL cells. PDL Cells were transfected with plasmids containing *hTERT*. Single cell cloning was then performed using a limiting dilution method. Reverse transcriptase polymerase chain reaction and stretch PCR were used to detect *hTERT* expression in clones. To determine whether clones could differentiate into osteoblasts, we stimulated cells with ascorbic acid and β -glycerophosphate.

3 Results and Discussion

Previously, Fujita et al. established human PDL cell line derived from permanent tooth utilizing *hTERT* gene alone [3]. Fujii et al. also succeeded in establishing human PDL cell lines derived from permanent tooth by transduction with *hTERT* and simian virus 40 (SV40) large T antigen (LT) [2]. Although immortalized PDL cells derived from human permanent tooth have been reported, immortalized PDL cells from deciduous tooth have not been isolated because exfoliated deciduous teeth lack their tooth root. In this study, we established clonal immortalized PDL cells derived from human deciduous teeth and described osteogenic properties.

We successfully obtained three single cell clones. All SH cells showed *hTERT* expression and stable proliferation after more than 80 population doublings and expressed marker genes of PDL cells, i.e. scleraxis, periostin, cementum protein 23, and tenomodulin. All clones expressed osteoblastic markers such as runt-related transcription factor-2 (RUNX-2), osterix, osteocalcin (OSC), osteonectin (OSN), type I collagen (Col I), alkaline phosphatase (ALP), parathyroid hormone receptor (PTHrP) but only clones from the SH 9 cell line differentiated into osteoblastic cells. To our knowledge, this is the first report of immortalization of PDL cells derived from human deciduous teeth [4]. These cells will be useful in studies investigating the cellular mechanisms and regenerative processes of human PDL.

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Pulpal Blood Flow Measurement Using a Laser Doppler Flowmetry Modified for Very Slow Blood Flow Velocity

Motohide Ikawa, Xiaofu Qu, Hideji Komatsu, and Hidetoshi Shimauchi

Abstract. The application of laser Doppler flowmetry (LDF) to detect pulpal blood flow (PBF) in aged adults has been difficult. An LDF that had a lower frequency range than conventional instrument has been developed for measuring very slow speed blood flow. The LDF for very low speed of blood flow detected signals from the teeth from which standard LDF did not detect obvious signals. The LDF for measuring very slow speed blood flow is considered to offer promise as a detection method of PBF in adult subjects.

Key words. Blood flow, Laser Doppler, Pulp, Tooth

1 Introduction

The laser (Doppler) tissue blood flowmeters (LDF) are used for measuring tissue blood flow of skin, brain and internal organs. Conventional LDF is designed to be able to measure almost every organ's blood flow. LDF measurement of the human tooth pulp has been applied as one of noninvasive pulp vitality testing [1]. When the conventional LDF is used for measuring a very low blood flow tissue such as dental pulp, obtained signals often cannot be used for the judgment if the blood flow changes or not. Because LDF shows only the last digit on the display changes; the

M. Ikawa (✉), X. Qu, and H. Shimauchi
Division of Periodontology and Endodontology, Department of Oral Biology,
Tohoku University Graduate School of Dentistry, 4-1 Seiryomachi,
Aoba-ku, Sendai 980-8575, Japan
e-mail: ikawa@dent.tohoku.ac.jp

H. Komatsu
Division of Pediatric Dentistry, Department of Oral Health
and Development Sciences, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

full range of the instrument is usually about 100 mL/min/100 g, and LDF in PBF measurement shows at most around 10 mL/min/100 g.

Ikawa et al. [2] reported age-related decrease of pulpal blood flow (PBF) signals obtained by LDF. In their report the signals recorded from the subjects of their ages over 40 years were almost at the level of the instrument's resolution limit. Therefore, the application of LDF to detect PBF in aged adults has been difficult.

2 Methods and Results

An LDF that had a lower frequency range than conventional instrument has been developed for measuring very slow speed blood flow was prepared. A comparison was made between the signals obtained from human upper central incisors using conventional LDF and the new LDF for measuring very slow speed blood flow.

The LDF for very low speed of blood flow detected signals from the teeth from which the standard LDF did not detect obvious signals. Significantly larger blood-flow signals were obtained from the new LDF than those from the conventional one.

3 Discussion

Results indicate that the blood flow velocity at the detection area in human upper central incisors in adults is too slow for the standard LDF to detect the movement of blood cells in the pulp. This is considered to be due to the reason; the velocity of blood cell at peripheral area in the pulp of adult subjects is decreased due to the calcified changes with age and the blood flow velocity is slower than other body tissues such as skin and muscle. The LDF for measuring very slow speed blood flow is considered to offer promise as a detection method of PBF in adult subjects. Comparisons between the vital and non-vital teeth will be required to determine the sensitivity and specificity in pulp vitality testing.

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Characterization of STRO-1 Positive Cells Derived from Human Wisdom Tooth Germs

Yoko Iwamatsu-Kobayashi, Daisuke Nishihara, Naoki Endo,
Koji Kindaichi, Junko Kindaichi, and Masashi Komatsu

Abstract. Recently, the research on the stem cells is developing rapidly in the odontology. Mesenchymal stem cells (MSCs) have the possibility useful for the stem cell treatment and the tissue engineering. STRO-1 is one of the mesenchymal stem cell markers. In this study, we examined the characterization of STRO-1 positive cells derived from human wisdom tooth germs (hWTGs) compared with STRO-1 negative cells. As positive control, we used human mesenchymal stem cells (HMSC) on purchase. STRO-1 positive cells derived from hWTGs showed Alcian blue positive matrix like HMSC when cultured in chondrogenic induction medium. STRO-1 negative cells from hWTGs did not show such matrix. Nodule formation stained with Alizarin red was performed not only by STRO-1 positive cells but also STRO-1 negative cells cultured in osteogenic induction medium. It is suggested that STRO-1 positive cells derived from hWTGs are useful for the stem cell treatment and the tissue engineering.

Key words. Chondrogenic induction, Human wisdom tooth germ, Mesenchymal stem cell, Osteogenic induction, STRO-1

Y. Iwamatsu-Kobayashi (✉), D. Nishihara, and M. Komatsu
Division of Operative Dentistry, Tohoku University Graduate School of Dentistry,
Sendai 980-8575, Japan
e-mail: iwa@m.tohoku.ac.jp

N. Endo
Division of Comprehensive Dentistry, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

K. Kindaichi
Division of Oral and Craniofacial Anatomy, Tohoku University
Graduate School of Dentistry, Sendai 980-8575, Japan

J. Kindaichi
National Center for Child Medical Health and Development, Tokyo 157-8535, Japan

1 Introduction

MSCs have the possibility useful for the stem cell treatment and the tissue engineering. They have been isolated from the dental pulp and periodontal ligament (PDL). STRO-1 is one of the mesenchymal stem cell markers. We have already reported that collection rate, alkaline phosphatase activity and protein content of STRO-1 positive cells from WTGs were higher than those from PDL [1]. In this study, we examined the characterization of STRO-1 positive cells derived from human wisdom tooth germs.

2 Materials and Methods

After informed consent, hWTGs tooth germs were extracted from healthy subjects (aged 8–12 years) by orthodontic reason. They were harvested with collagenase and disperse to dissociate into cells and cultured in mesenchymal stem cells growth medium (MSCGM) (Lonza, Walkersville, MD, USA). Cells were used for the examination from passage 3 to 5. STRO-1 positive cells were isolated by immunomagnetic bead selection (Dynabeads®, Invitrogen, Carlsbad, CA) using the STRO-1 (MAB1038 R&D System, Minneapolis, MN) antibody. Bead-negative cells were also collected and used for the examination. For positive control, Poietics® human mesenchymal stem cells (HMSC) (Lonza, Walkersville, MD, USA) on purchase were used.

Chondrogenic induction was performed using chondrogenic induction medium (Lonza, Walkersville, CA) according to the manufacturer's recommendation. Briefly, 5×10^5 cells were centrifuged and cell pellets were fed the complete chondrogenic medium containing TGF- β 3 for 4 weeks. Then pellets were fixed with 4% paraformaldehyde and paraffin sections were stained with Alcian blue (pH 2.5). Hyaluronidase digestion was performed for 2 h before Alcian blue staining.

Osteogenic induction was performed using osteogenic induction medium (Lonza, Walkersville, CA). Briefly, cells were seeded 1.5×10^4 cells/ml in a 24-well plate in MSCGM. After 24 h, the medium was transitioned to osteogenesis induction medium containing dexamethasone, L-glutamine, ascorbate and β -glycerophosphate for 4 weeks. Then specimen were fixed with 4% paraformaldehyde and Alizarin red S staining was performed.

3 Results

STRO-1 positive cells derived from hWTGs showed Alcian blue positive matrix when cultured in chondrogenic induction medium. The matrix was digested with hyaluronidase like positive control using HMSC. They also performed nodule

formation stained with Alizarin red as HMSC cultured in osteogenic induction medium. Nodule formation was performed not only by STRO-1 positive cells but also by negative cells.

4 Conclusion

It is suggested that STRO-1 positive cells derived from hWTGs are useful for the stem cell treatment and the tissue engineering.

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The Influence of Mesenchymal Stem Cell Growth Medium for Isolation of STRO-1 Positive Cells from Human Periodontal Ligament

Naoki Endo, Yoko Iwamatsu-Kobayashi, Daisuke Nishihara,
Masaaki Iwamatsu, Masashi Komatsu, and Masahiko Kikuchi

Abstract. In this study, we examined the influence of mesenchymal stem cells growth medium (MSCGM) for STRO-1 positive cells derived from periodontal ligament (PDL) compared with DMEM.

PDL cells cultured in DMEM showed spindle shape. On the other hand, PDL cells cultured in MSCGM for 7 days showed colony formation. The isolation rate of STRO-1 positive cells in MSCGM was 1.57 times higher than that in DMEM in 7 days culture and 2.49 times higher in 14 days culture.

It is suggested that MSCGM is effective to increase the isolation rate of STRO-1 positive cells from PDL.

Key words. Immunocytochemistry, Isolation rate, Mesenchymal stem cells, Periodontal ligament, STRO-1

1 Introduction

STRO-1 identifies a cell-surface antigen expressed by a small subset of mesenchymal stem cells (MSCs). It was reported that MSCs which had the high differentiation and proliferation potency were separated from PDL and expressed STRO-1.

We have already reported that the isolation rate of STRO-1 positive mesenchymal stem cells from periodontal ligament (PDL) was lower than that from human wisdom tooth germs [1].

N. Endo (✉), M. Iwamatsu, and M. Kikuchi
Division of Comprehensive Dentistry, Tohoku University Hospital,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: y-n-o@m.tohoku.ac.jp

Y. Iwamatsu-Kobayashi, D. Nishihara, and M. Komatsu
Division of Operative Dentistry, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

In this study, we examined the influence of MSCGM for STRO-1 positive cells derived from PDL compared with DMEM.

2 Materials and Methods

Human PDL cells were obtained from clinically healthy third molars (aged 20–31 years). Informed consent was obtained from all patients. Healthy periodontal tissue was removed from middle third of the root surface and transferred to culture dishes. Cells were cultured in DMEM (Sigma, St. Louis, MO, USA) supplemented with 10% FBS and used for the examination from passage 3 to 5.

PDL cells were plated at $1 \times 10^5/\text{mL}$ in 6-well dishes and cultured 7 and 14 days in MSCGM (Thera PEAK® MSCGM-CD®) (Lonza, Walkersville, MD) or DMEM. After cultured in MSCGM or DMEM, some specimens were fixed by immersion in 4% paraformaldehyde. They were incubated with the monoclonal mouse IgM anti human STRO-1 antibody (MAB1038) (R&D systems Inc., Minneapolis, MN) and Rhodamin-conjugated with secondary antibody. Staining was visualized under a Keyence PZ-9000 fluorescence microscope.

The mesenchymal stem cells were isolated by immunomagnetic bead selection (Dynabeads®) (Invitrogen, Carsbad, CA) using the STRO-1 antibody. The number of isolated cells was counted by hemacytometer and collection rate was calculated.

3 Results

PDL cells cultured in DMEM for 7 days were elongated and showed spindle shape. On the other hand, PDL cells cultured in MSCGM for 7 days showed colony formation.

After 14 days, PDL cells in MSCGM were elongated. Some specimens cultured in MSCGM for 14 days were detached from culture dish as cell sheets. Those in DMEM for 14 days were not detached.

The isolation rate of STRO-1 positive cells were in MSCGM was 1.57 times higher than that in DMEM in 7 days culture and 2.49 times higher in 14 days culture.

4 Discussion

MSCGM used in this study is a serum free, chemically defined medium for the growth of human MSCs. It is said to be optimized for the multiple passage expansion of all types of human MSCs while still allowed for differentiation into the several desired lineages.

In this study, PDL cells could be directly transitioned from serum-containing DMEM to MSCGM with little to no adaptation time.

PDL cells cultured in MSCGM showed morphological difference like colony formation from those in DMEM.

The isolation rate of STRO-1 positive cells was higher than that in DMEM.

Those data suggests that PDL cells cultured in MSCGM is effective to obtain STRO-1 positive cells and may contribute to the tissue engineering for dental area.

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IL-12-Mediated and IL-18-Mediated Nitric Oxide-Induced Apoptosis of Adherent Bone Marrow Cells in TNF- α -Induced Osteoclast Formation

Hideki Kitaoura, Tomo Aonuma, Emiko Fukumoto, Keisuke Kimura, Toshiya Fujii, Zaki Weli Hakami, and Teruko Takano-Yamamoto

Abstract. It has recently been reported that TNF- α has the ability to accelerate osteoclastogenesis. We previously reported that the proinflammatory cytokine IL-12 and IL-18 induced apoptosis in TNF- α -mediated osteoclastogenesis in mouse bone marrow culture through an interaction of Fas and Fas ligand (FasL). However, the inhibition of Fas-FasL interaction by using anti-FasL antibody could not completely inhibit apoptosis. Therefore, it is possible that IL-12 and IL-18 may also trigger some other apoptotic mechanisms. Nitric oxide (NO) may act as a mediator of the apoptotic effect. We found that NO production was induced in bone marrow cells cultured with IL-12 and IL-18 in the presence of TNF- α . Apoptosis was partially inhibited when bone marrow cells were treated with NO inhibitors. The simultaneous effects of TNF- α and IL-12 or IL-18 on bone marrow cells induce apoptosis by the production of NO.

Key words. Apoptosis, IL-12, IL-18, Nitric oxide, Osteoclast, TNF- α

1 Introduction

Formation of mature osteoclasts is dependent on macrophage-colony-stimulating factor (M-CSF) and a ligand for the receptor activator of necrosis factor κ B (RANKL). M-CSF is indispensable for proliferation and differentiation of osteoclast precursors. It has been reported that TNF- α has also been recognized as an

H. Kitaoura (✉), T. Aonuma, E. Fukumoto, K. Kimura, T. Fujii, Z.W. Hakami, and T. Takano-Yamamoto
Division of Orthodontics and Dentofacial Orthopedics, Tohoku University
Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: hkitaura@m.tohoku.ac.jp

important factor in osteoclastogenesis. TNF- α -induced osteoclast recruitment is probably central to bone erosive diseases, such as rheumatoid arthritis, periodontal disease and periprosthetic bone loss. The course of osteoclast formation can be controlled by local factors, including cytokines and prostaglandins, and systemic factors such as sex steroids. The cytokines interleukin (IL)-6, IL-17, transforming growth factor (TGF)- β and TNF- α induce osteoclast formation and increase bone-resorbing activity of osteoclasts. In contrast, IL-4, IL-10, IL-12, IL-13, IL-18 and interferon (IFN)- γ inhibit osteoclast formation and decrease several activities of osteoclasts [1].

2 IL-12 and IL-18 Induced Apoptosis

It has been reported that osteoclastogenesis induced by RANKL decreased in the presence of IL-12 in vitro. In addition, TNF- α -mediated osteoclastogenesis was inhibited by the induction of apoptosis mediated by the interaction of IL-12-induced FasL and TNF- α -induced Fas [2]. Furthermore, IL-18 inhibits TNF- α -mediated osteoclastogenesis in bone marrow cell cultures by Fas/FasL interaction. Finally, IL-12 and IL-18 inhibited TNF- α -mediated osteoclastogenesis by up-regulating FasL synergistically [3]. Nitric oxide (NO) has been related to various physiological and pathological conditions. NO is believed to serve two functions in cell destiny: excessive levels of NO cause cytotoxicity, while low levels may stimulate cell survival. Inhibitory effects of NO on osteoclastic bone resorption have been demonstrated. It is believed that osteoclast bone resorption associated with inflammatory diseases may be influenced by local NO levels in the pathologic region because NO induction commonly occurs in inflammatory conditions. We reported that NO production was induced in bone marrow cells cultured with IL-12 and IL-18 in the presence of TNF- α . Apoptosis was partially inhibited when bone marrow cells were treated with NO synthase inhibitors [4].

3 Conclusion

In summary, we found that IL-12 and IL-18 induced NO production in bone marrow cells cultured with TNF- α . IL-12 and IL-18 up-regulated NO production in the presence of TNF- α . Furthermore, the apoptosis was partly inhibited when bone marrow cells were treated with NO synthase inhibitors. These results suggested that the simultaneous effects of TNF- α and IL-12 or IL-18 on bone marrow cells induce apoptosis, and that the apoptosis is partly caused by the production of NO.

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Measurement of Tissue Oxygenation Level in Human Lip

Hideji Komatsu, Motohide Ikawa, Keishiro Karita,
and Satoshi Fukumoto

Abstract. Kashima (Optics Laser Technol 35: 485–489, 2003) reported a method based on the Beer–Lambert law for measuring blood volume and its oxygenation in a small volume of tissue ($<1\text{ cm}^3$). In the present study, the applicability of a tissue blood oxygenation monitor based on Kashima's report (Optics Laser Technol 35: 485–489, 2003) to human lip was examined. The amount of oxy-hemoglobin, the amount of deoxy-hemoglobin, total amount of hemoglobin (THb) and oxygen saturation levels (StO_2) in human lower lips and in fingerpads of five healthy participants (age: 28–55) were measured using the tissue blood oxygenation monitor. Tissue oxygenation levels in lips ranged between 81% and 88%, while those in fingerpads ranged between 77% and 83%. Deep breath evoked a transient decrease of StO_2 both in lip and fingerpad, and the change in StO_2 was not synchronous either with BF or THb. The results indicated that the device used in this study reflected tissue oxygenation levels.

Key words. Fingerpad, Human lip, Tissue blood oxygenation

H. Komatsu (✉) and S. Fukumoto
Division of Pediatric Dentistry, Department of Oral Health and Development Sciences,
Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku,
Sendai 980-8575, Japan
e-mail: komatsu@dent.tohoku.ac.jp

M. Ikawa
Division of Periodontology and Endodontology, Department of Oral Biology,
Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku,
Sendai 980-8575, Japan

K. Karita
Division of Oral Diagnosis, Department of Oral Medicine and Surgery,
Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku,
Sendai 980-8575, Japan

1 Introduction

Kashima [1] reported a method based on the Beer–Lambert law for measuring blood volume and its oxygenation in a small volume of tissue ($<1\text{ cm}^3$). This method utilized the large differences between the absorption of oxy-hemoglobin and deoxy-hemoglobin by three red laser lights (630, 650, 690 nm). To our knowledge, there was no report that showed measurement of tissue oxygenated in human lip by Kashima's method [1]. Therefore, the applicability of the tissue oxygenation monitor based on Kashima's report [1] to human lip was examined.

2 Materials and Methods

The amount of oxy-hemoglobin, the amount of deoxy-hemoglobin, total amount of hemoglobin (THb) and oxygen saturation levels (StO_2) in human lower lips and fingerpads of five healthy participants ($n=5$, age: 28–55) were measured using the tissue blood oxygenation monitor. Blood flow (BF) of the tissue was simultaneously monitored using a laser Doppler flowmetry. Approximately 10 min were required to fulfill one recording session, and the subjects did not claim any discomfort during the measurement. Recordings were stored in a computer (iMac, Apple Computer Inc.) via a laboratory interface (MacLab/8s, ADInstruments Pty Ltd., Australia) and signal processing software (Chart, ADInstruments Pty Ltd., Australia). This study was approved by the Ethical Committee, Tohoku University Graduate School of Dentistry. The purpose and methods of the study was explained to subjects, and informed consent was obtained from all of them.

3 Results

Cessation of the deep breath evoked a transient decrease of StO_2 both in lip and fingerpad, and the change in StO_2 was not synchronous either with BF or THb. Tissue oxygenation levels in lips ranged between 81% and 88%, while those in fingerpads ranged between 77% and 83%. The significant difference of StO_2 between lips and fingerpads were obtained ($P=0.0431$, Wilcoxon signed-ranks test).

4 Discussion and Conclusion

Tissue oxygenation levels in lips were higher than fingerpads. High development of arteriovenous anastomosis (AVA) in lips are recognized to increase the high ratio of arterial blood.

It is suggested that decrease of the tissue oxygenation level after cessation of the deep breath shows the responses evoked by the decrease of the oxygen supply at the transient cessation of the respiration. The results indicated that the device used in this study reflected tissue oxygenation levels.

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Effects of the Small Molecule Dorsomorphin on Intracellular Signaling

Tada-aki Kudo, Hiroyasu Kanetaka, Kazutoshi Mizuno,
Yasuhiro Ryu, Ye Zhang, Mitsuhiro Kano, Yoshinaka Shimizu,
and Haruhide Hayashi

Abstract. The small molecule dorsomorphin, also known as compound C, is a cell-permeable pyrazolopyrimidine derivative, which acts as a potent and reversible ATP-competitive inhibitor of AMP-activated protein kinase (AMPK). Recent studies indicated that several molecules with functions in other signaling pathways, including bone morphogenetic protein (BMP) signaling, are novel targets of dorsomorphin. This report focuses on two major targets of dorsomorphin and provides insight into the use of dorsomorphin in the analysis of intracellular signaling.

Key words. AMP-activated protein kinase (AMPK), Bone morphogenetic protein (BMP), Dorsomorphin

T. Kudo (✉), K. Mizuno, Y. Ryu, and H. Hayashi
Division of Oral Physiology, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: tkudo@m.tohoku.ac.jp

H. Kanetaka and Y. Zhang
Liaison Center for Innovative Dentistry, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

M. Kano
Division of Oral and Craniofacial Anatomy, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

Y. Shimizu
Division of Oral Pathology, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

1 Introduction

AMP-activated protein kinase (AMPK), a conserved cellular energy sensor, is a key “fuel gauge” to maintain the intracellular as well as body energy balance. It stimulates energy-producing processes, such as glucose uptake, fatty acid oxidation, and glycolysis, and inhibits energy-consuming processes, such as lipogenesis, protein synthesis, and gluconeogenesis [1]. Bone morphogenetic proteins (BMPs) bind to heterogeneous complexes of kinase receptors known as type I and II BMP receptors (BMPRI and BMPRII, respectively) and elicit diverse cellular responses. BMPRII phosphorylates BMPRI, which activates Smads through phosphorylation, and they in turn upregulate the expression of target genes such as *Id1* [2, 3]. Dorsomorphin has been used experimentally to attenuate AMPK activity, but it was recently identified in a phenotypic screen for compounds that perturb dorsoventral axis formation by inhibiting BMP signaling in zebrafish [2]. This compound is now used for studying the molecular mechanisms that promote metabolism, pluripotent self-renewal, and differentiation, as described below.

2 Inhibition of AMPK Signaling by Dorsomorphin

Dorsomorphin is a highly selective AMPK inhibitor, and does not significantly inhibit several structurally related kinases, including protein kinase C, protein kinase A, and Janus kinase 3 [1]. For example, a recent study showed that dorsomorphin treatment significantly inhibited adipogenic differentiation of mouse 3T3-L1 fibroblasts by suppressing AMPK activity [1]. In addition, dorsomorphin was shown to reverse the anti-proliferative effect of AMPK signaling in glucose-deprived mouse neural stem cells by inhibiting AMPK activity [4].

3 Inhibition of BMP Signaling with Dorsomorphin

Dorsomorphin was identified as an inhibitor of Smad 1/5/8 phosphorylation by BMP type I receptors ALK2, ALK3, and ALK6 [2]. This inhibition was shown to reduce heterotopic ossification, as well as to decrease BMP-regulated hepatic hepcidin gene transcription that leads to increased iron levels in vivo [5, 6]. Dorsomorphin treatment of mouse pluripotent embryonic stem cells (ESCs) efficiently blocked activation of the BMP-target gene *Id1* and induced cardiomyogenesis [7]. Inhibition of endogenous BMP signaling by dorsomorphin also accelerated and enhanced myogenic differentiation in C2C12 myoblasts, similar to the BMP antagonist Noggin [8]. Recent studies also showed that dorsomorphin promotes self-renewal as well as neural induction of human ESC by inhibiting BMP signaling [9, 10].

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Expression of Aryl Hydrocarbon Receptor in Bone Tissues

Yasuhiro Miki, Shuko Hata, Ryoko Saito, Katsuhiko Ono,
Hironobu Sasano, and Hiroyuki Kumamoto

Abstract. Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor responsive to environmental compounds such as dioxin. AhR signal has been known as xenobiotic receptor or xenobiotic sensor. Recently, the roles of AhR through the induction of cytokines come into focus. In bone tissues, AhR and its signaling pathway are detected in both osteoblasts and osteoclasts. In vitro and in vivo studies have been suggested that AhR signal mediates osteogenesis via cytokine signal in human bone as well as experimental animals. In this short review, we focused on the importance of AhR in bone and hard tissues.

Key words. Aryl hydrocarbon receptor, Dioxin, Osteoblast, Osteoclast, Osteogenesis

1 Aryl Hydrocarbon Receptor

The aryl hydrocarbon receptor (AhR) is activated by a variety of compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 3-methylcholanthrene (3-MC) [1]. The ligand-bound AhR translocates to the nucleus and heterodimerizes with AhR nuclear translocator (Arnt) [1]. The AhR/Arnt heterodimer binds to a specific sequence within the promoter lesion of various target genes designated a xenobiotic

Y. Miki (✉) and H. Kumamoto

Division of Oral Pathology, Department of Oral Medicine and Surgery,
Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai, Miyagi 980-8575, Japan
e-mail: miki@patholo2.med.tohoku.ac.jp

S. Hata, R. Saito, K. Ono, and H. Sasano

Division of Anatomic Pathology, Department of Pathology,
Tohoku University Graduate School of Medicine,
2-1 Seiryomachi, Aoba-ku, Sendai, Miyagi 980-8575, Japan

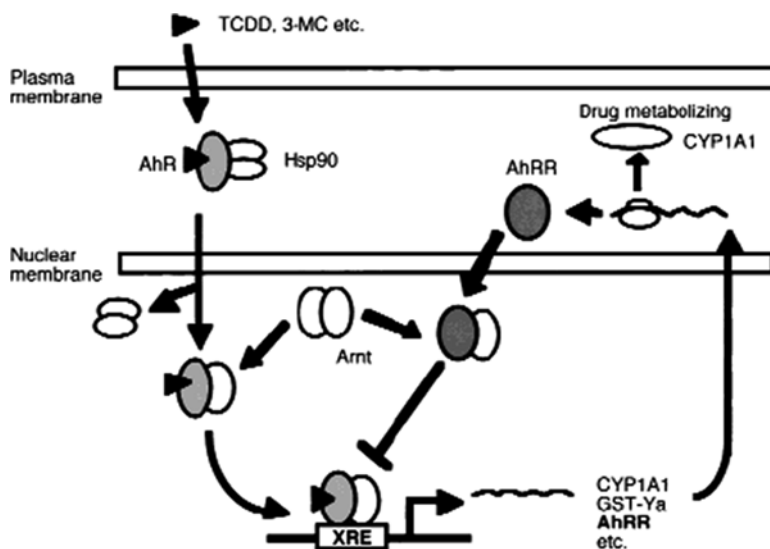


Fig. 1. AhR ligands: *TCDD* 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, *3-MC* 3-methylcholanthrene (3-MC). Molecular chaperone: *Hsp90* heat shock protein 90. Heterodimer partner: *Arnt* AhR nuclear translocator. Cis-element: *XRE* xenobiotic responsive element. Target genes: *CYP1A1* cytochrome P450 1A1, *GST-Ya* glutathione S-transferase Ya subunit, *AhRR* AhR repressor (AhRR inhibits AhR function by competing with AhR for heterodimerizing with Arnt [1]). Reproduced from [1] with permission of *Gene & Development*

responsive element (XRE) to activate their expression [1]. The ligand-activated AhR/Arnt mediates transcriptional activation of cytochrome P450 (CYP) 1A, CYP1B, etc. The AhR signal is summarized in Fig. 1 [1]. Recently, the roles of AhR in immune or inflammatory system through the induction of cytokines such interleukin (IL) -1, IL-6, IL-18, etc., which have XRE sequences in their promoters [1], come into focus.

2 AhR and Bone

The activity of alkaline phosphatase and the expression level of osteocalcin were decreased by treatment of 3-MC in osteoblast (like) cells derived from rodents [2]. TCDD significantly decreased the mRNA levels of RUNX2, alkaline phosphatase and osteocalcin in mesenchymal stem cells derived from bone marrow of rodents [3]. TCDD also significantly decreased the number of osteoclasts and the area of bone resorption [3]. In vivo analysis, AhR protein was detected in both rat osteoblasts and osteoclasts but not osteocytes [2]. The treatment of 3-MC to pregnant mice and fetuses decreased the appropriate calcification of bones [2]. In dental hard tissues, the relationship between dioxin derived from mother's milk and developmental

mineralization defects in permanent first molars was also detected in healthy Finnish children born in the late 1980s [4]. These findings all suggested that AhR might have critical effects on the development of bone and dental hard tissues.

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Deformation Distribution Analysis of Alveolar Bone Model in the Vicinity of a Dental Implant Using a Digital Image Correlation Method

Yasuyuki Morita, Mitsugu Todo, Yasuyuki Matsushita,
and Kiyoshi Koyano

Abstract. Deformation distribution of alveolar bone model around a dental implant was measured by using an advanced experimental technique, digital image correlation method (DIC). The strain distributions under simulated dental occlusion were visualized not only qualitatively, but also quantitatively. Then, it was elucidated that the advanced experimental method, DIC, enabled to evaluate the safety and reliability of dental implantology.

Key words. Bone model, Dental implant, Digital image correlation, Strain distribution, Whole-field measurement

1 Background

It is important to reveal deformation distribution of alveolar bone around dental implants since mechanical milieu causes majority of bone resorption and implant fracture in implant dentistry. However in fact, the clarification was conducted only by numerical techniques, such as finite element analysis (FEA), because of difficulty of the experiments. The numerical evaluation always requires clinical

Y. Morita (✉)

Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku,
Nagoya 464-8603, Japan
e-mail: morita@mech.nagoya-u.ac.jp

M. Todo

Research Institute for Applied Mechanics, Kyushu University,
6-1 Kasuga-koen, Kasuga 816-8580, Japan

Y. Matsushita and K. Koyano

Graduate School of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku,
Fukuoka 812-8582, Japan

and experimental verification. Therefore, although experimental perceptions are important, existing experimental techniques in this study field, such as strain gages and photoelasticity, do not satisfy the demands of the experimental clarification.

2 Objective

The deformation distribution behavior of the alveolar bone around dental implants under dental occlusion is elucidated by using an advanced experimental technique, a digital image correlation method.

3 Method

SAWBONES laminated test blocks, which have same bilayer structure as actual alveolar bone, were employed as the bone model specimen. Therefore, the bone model specimen consists of cortical and cancellous bone layers. Then, simulated dental occlusion force was applied to the specimen which inserted an actual fixture (see Fig. 2a). A digital image correlation method was performed to visualize the deformation distribution behavior quantitatively.

4 Results

The load–displacement curve of the specimen, shown in Fig. 1, was obtained the same results of actual occlusion tests. The load was increased linearly with the displacement. The strain distribution behaviors were shown in Fig. 2. Then, we visualized that the maximum ϵ_{xx} and γ_{xy} strains were concentrated in the bone at the tapered

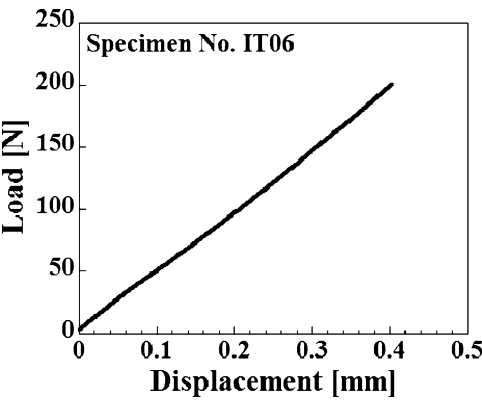


Fig. 1. Load–displacement curve of the specimen during simulated dental occlusion

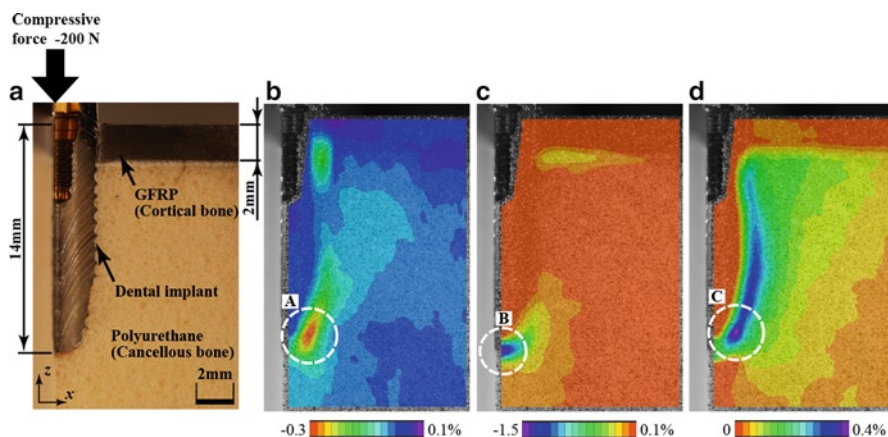


Fig 2. Strain distributions under simulated dental occlusion at -200N . (a) Specimen configuration and observation area. (b) ϵ_{xx} field. (c) ϵ_{zz} field. (d) γ_{xz} field

position of the implant (see circles A and C in Figs. 2b, d, respectively). The maximum concentration of the ϵ_{yy} appeared in the bone at the tip of the implant (see circle B in Fig. 2c). The values of the maximum ϵ_{xx} , ϵ_{yy} , and γ_{xy} strains were -0.30% , -1.5% , and 0.40% , respectively.

5 Conclusion

The applied experimental technique, digital image correlation method, in this paper made it possible to quantitatively obtain more realistic deformation distribution behaviors of alveolar bone around a dental implant compared with existing experimental methods.

Effects of Capsaicin Treatment on Nociception and Structure of Trigeminal Nerve Fibers in Adult Rats

Akiko Kato, Megumi Nakamura, Seishi Echigo, and Yasuyuki Sasano

Abstract. Systemic capsaicin treatment has been used in animal research to eliminate sensory nerve function, but little is known about physiological and morphological effects. We investigated the time-dependent changes in nociception and the ultrastructure of sensory nerve fibers after administration of capsaicin in rats. Capsaicin treatment caused transient loss of sensitivity, but transmission electron microscopy showed no structural changes of trigeminal nerve fibers in capsaicin-treated rats as well as controls. These results suggest that the structural damage to sensory nerve fibers may not be a cause of the reduction of sensitivity.

Key words. Adult rats, Capsaicin, Nociception, Transmission electron microscopy, Trigeminal nerve

A. Kato

Division of Oral Surgery, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
and

Division of Craniofacial Development and Regeneration, Tohoku University
Graduate School of Dentistry, Sendai 980-8575, Japan

M. Nakamura (✉) and Y. Sasano

Division of Craniofacial Development and Regeneration, Tohoku University
Graduate School of Dentistry, Sendai 980-8575, Japan
e-mail: megmeg-cbn@m.tohoku.ac.jp

S. Echigo

Division of Oral Surgery, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

1 Introduction

Systemic administration of capsaicin to adult rats has been known to induce loss of sensitivity of sensory neurons systemically. However, there is little information about the effects of capsaicin treatment on structure of sensory nerve fibers. It has been known that the loss of nociception is caused by depletion of neuropeptides contained in nociceptive neurons such as substance P and calcitonin gene related peptide (CGRP). Effects of capsaicin treatment on structure of sensory nerve fibers are controversial. Some claimed that capsaicin treatment damages the structure of sensory nerve fibers [1, 2], while others reported no structural damage to them [3, 4]. In this study, we examined the time-dependent changes in nociception with capsaicin treatment. The effect of capsaicin on the ultrastructure of sensory nerve fibers was also evaluated.

2 Materials and Methods

Nine-week-old male Wistar rats were used. Capsaicin was dissolved in vehicle (10:10:80 of Tween-80, ethanol, saline) to 10 mg/mL. The rats received subcutaneous injections of capsaicin or vehicle after an intraperitoneal injection of sodium pentobarbital (40 mg/kg) and atropine (0.2 mg/kg). The total capsaicin dose (125 mg/kg) was administered as a series of three injections (2.5, 5.0, 5.0 mL/kg). All three injections were done within one day: in the morning, the afternoon and the next morning. Their nociceptive level was assessed time-dependently using eye wiping test. Briefly, a drop of 5M NaCl solution was put into the right or left eye of rats. The number of eye wipes with the forepaws was counted during 30 s. Ten rats in each of the control and capsaicin-treated group were examined before treatment, and at 1, 2, 3, 4 and 6 weeks after treatment. Data obtained from the eye wiping test were statistically analyzed using SPSS 16.0 J for Windows (SPSS Japan Inc, Tokyo, Japan). Wilcoxon signed rank test was used, followed by the Bonferroni correction to compare within each group. The Mann-Whitney U test was used to compare between the two groups. $P < 0.05$ was considered statistically significant.

The trigeminal nerve was resected from rats perfusion-fixed at 2, 4 and 6 weeks after administration of capsaicin or vehicle. The specimens were examined morphologically with transmission electron microscopy.

3 Results

In capsaicin-treated rats, loss of nociception was occurred transiently. The reduction of nociception with systemic capsaicin treatment continued for 4 weeks. The sensitivity was most reduced at Week 1 after administration of capsaicin and recovered

by Week 6. There was no significant difference among controls. Transmission electron microscopy showed no structural changes in trigeminal nerve fibers of capsaicin-treated rats compared to controls.

4 Conclusion

The present study confirmed that systemic capsaicin treatment for adult rats induce transient loss of nociception. Our results also suggest that the structural damage to sensory nerve fibers may not be a cause of the reduction of sensitivity.

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Extracellular Phosphate Increases Bone Morphogenetic Protein-2 Expression in Human Dental Pulp Cells and Human Periodontal Ligament Cells

Eiji Nemoto, Hiroyuki Tada, and Hidetoshi Shimauchi

Abstract. Extracellular phosphate (Pi) has been shown to act as a signaling molecule capable of altering gene expression and cellular function during osteoblastic differentiation. In this study, we investigated whether Pi regulates expression of bone morphogenetic protein (BMP)-2, a crucial regulator of osteogenic differentiation, and explored the signaling pathway involved using human dental pulp cells and human periodontal ligament cells. We found that Pi increased BMP-2 gene expression. Experiments using signaling inhibitors revealed that the increase in *BMP-2* expression was dependent on the cAMP/protein kinase A signaling pathway. We also showed that Pi activates the ERK1/2, and treatment with PD98059, an ERK1/2 inhibitor, suppressed Pi-mediated BMP-2 increase, indicating that activation of the ERK1/2 pathway is required for this Pi-mediated response. These findings demonstrate a novel ability of Pi to stimulate BMP-2 via cAMP/protein kinase A and ERK1/2 pathways.

Key words. BMP-2, Phosphate

1 Introduction

Inorganic phosphate (Pi) is essential for extracellular matrix mineralization and plays an important role in the development and activity of osteogenic cells. However, specific signaling pathways involved in the regulation of gene expression in response to Pi have not been well elucidated. The bone morphogenetic proteins (BMPs) are structurally related to the TGF- β superfamily and were originally identified by their

E. Nemoto (✉), H. Tada, and H. Shimauchi
Department of Periodontology and Endodontology, Tohoku University Graduate
School of Dentistry, 4-1 Seiryō-machi, Aoba, Sendai 980-8575, Japan
e-mail: e-nemoto@umin.ac.jp

capacity to induce ectopic bone formation in rodents. Among the BMP family members, BMP-2 has been studied extensively for its various biological functions, particularly during osteogenic differentiation. Forced expression of BMP-2 in mesenchymal stem cells and osteoblasts enhances osteogenic differentiation of these cells *in vitro* and bone formation. These findings indicate that BMP-2 may be a therapeutic agent for diseases related to not only bone loss, but also dentin loss. In this study, we examined the possible regulation of BMP-2 expression in human dental pulp cells and periodontal ligament cells in response to extracellular Pi. We demonstrated that Pi has the ability to increase BMP-2 gene expression in both cells, and that activation of the cAMP/protein kinase A (PKA) signaling pathway and the ERK1/2 pathway is involved in Pi-mediated BMP-2 expression [1].

2 Results and Discussion

The gene expression level of BMP-2 in human dental pulp cells and human periodontal ligament cells increased upon stimulation with 3 mM Pi for 48 h. Pi-mediated increase in BMP-2 was completely and partially inhibited by pretreatment with adenylate cyclase inhibitor (MDL-12,330A) and PKA inhibitor (H-89), respectively, indicating that cAMP/PKA signaling is involved in Pi-mediated BMP-2 regulation. Treatment with Pi resulted in biphasic activation of ERK1/2. The ERK1/2 activation was initiated within the first 15 min and returned to basal levels by 1 h, and the activation was detectable again after 3 h and enhanced at least through 24 h. Furthermore, pretreatment of cells with an inhibitor of MEK1/2, PD98059 significantly inhibited the increase in BMP-2 mRNA in response to Pi, suggesting that activation of the ERK1/2 signaling pathway is required for Pi-mediated BMP-2 mRNA increase. These findings and mechanistic analysis of extracellular Pi modulation of BMP-2 expression in cells responsible for hard tissue regeneration should assist in the design of regenerative therapies for dentin and periodontal tissues on the basis of sound biological principles.

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In Vitro Adherence of *Candida albicans* to Acrylic Resin with Different Surface Status

Taro Nomura, Tetsuya Suzuki, Junichi Furuya, Yu Shimoyama, Minoru Sasaki, and Shigenobu Kimura

Abstract. This study aimed to investigate quantitatively the adhesion of *Candida albicans* to three clinically-used acrylic resins with different surface status. All the cuboid-shaped resin specimens were sterilized, and the some were kept in sterilized PBS hours at RT. *C. albicans* IFO 1385 cells were placed on the specimen and incubated for 1 h at 4°C. After washing and staining, the adhered *C. albicans* cells were counted with a digital microscope. The results indicated that markedly lower number of *C. albicans* adhered to the polished specimens than the unpolished ones regardless of the polishing method, and no marked difference was found among the three types of resin tested. In the same surface texture, less *C. albicans* cells were observed on the wet specimens. Taken together, the keeping of the acrylic denture surfaces finely-polished and wet-conditioned could provide a useful approach toward the reduction of *C. albicans* adherence.

Key words. Acrylic resins, Adherence, *Candida albicans*, Surface status

1 Introduction

Candida albicans is one of the major causative microorganisms of denture stomatitis. However, the surface status of the resin to which *C. albicans* adhere has not been fully elucidated. In this study, we investigated quantitatively the adhesion of

T. Nomura (✉), T. Suzuki, and J. Furuya
Division of Removable Prosthodontics, Iwate Medical University
School of Dentistry, 1-3-27 Chuodori, Morioka, Iwate 020-8505, Japan
e-mail: tanomura@iwate-med.ac.jp

Y. Shimoyama, M. Sasaki, and S. Kimura
Division of Molecular Microbiology, Iwate Medical University
School of Dentistry, Iwate, Japan

C. albicans to the three acrylic resins with different surface textures (polished/unpolished) and conditions (wet/dry).

2 Materials and Methods

Three acrylic resins [heat-curing resin; ACRON[®], pour resin; PROCAST[®], and self-curing repair resin; UNIFASTIII[®] (GC Co., Japan)] were given cuboid shapes (1.0×1.0×0.2 cm). The specimens were polished, and the surface roughness (R_a) was measured. After sterilization, some were kept in sterilized PBS up to 48 h at RT. *C. albicans* IFO 1385 (1×10^4) were then placed on a specimen and incubated for 1 h at 4°C. After incubation, the adhered *C. albicans* cells were counted by a digital microscope at ×400 magnification.

3 Results and Conclusions

Markedly lower number of *C. albicans* adhered to the polished specimens. A linear regression analysis indicated a significant positive correlation between the R_a and the adherent number of *C. albicans* (Fig. 1), suggesting that *C. albicans* adhere preferably to the unpolished resin surface. Further, the adherent number of *C. albicans* under wet condition was relatively lower than that under dry condition in the same R_a . In the same surface texture, however, no significant difference was found among the three types of resin. Thus, the present findings suggested that keeping the acrylic denture surfaces finely-polished and wet-conditioned, regardless of the type of resin, could provide a useful approach toward the reduction of *C. albicans* adherence.

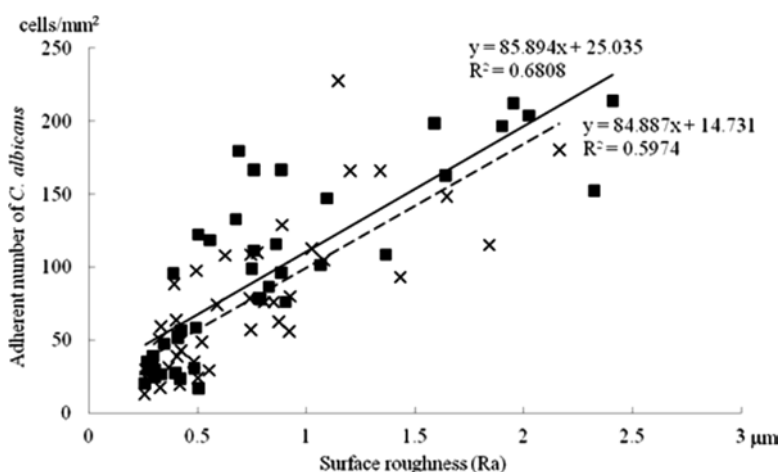


Fig. 1. The relationship between the R_a and the adherent number of *C. albicans*. Solid and dotted lines are the regression lines under dry (filled square) and wet (times) conditions, respectively

Root Elongation and Periodontal Tissue Formation in Tooth Germs Allogeneically Transplanted Between Rat Littermates

Koji Andou, Megumi Nakamura, Yoshinori Ina, Keiichi Sasaki,
and Yasuyuki Sasano

Abstract. This review focused on root elongation and periodontal tissue formation after allogenic tooth germ transplantation between rat littermates using micro-CT and histology. The upper right second molar germs in 2-week-old rats were extracted and immediately transplanted into the upper right first molar socket of rat littermates under anesthesia. Roots elongated after allogenic transplantation, but they were significantly shorter than control roots. The number of roots varied from 1 to 4 in transplanted teeth. Periodontal tissue formation in transplanted teeth was equivalent to that of the control teeth.

Key words. Allogenic transplantation, Micro-CT, Rats, Root development, Tooth

1 Introduction

Little information is available about root elongation and periodontal tissue formation in tooth germs allogeneically transplanted between rat littermates. Reports have shown low rejection rates of transplanted teeth after experimental allogenic transplantation

K. Andou

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
and

Division of Craniofacial Development and Regeneration,
Tohoku University Graduate School of Dentistry, Sendai 980-8575, Japan

M. Nakamura and Y. Sasano (✉)

Division of Craniofacial Development and Regeneration,
Tohoku University Graduate School of Dentistry, Sendai 980-8575, Japan
e-mail: sasano@anat.dent.tohoku.ac.jp

Y. Ina and K. Sasaki

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

between mouse littermates [1], which may provide a useful experimental model for analyzing root and periodontal tissue development in allogenic transplantation. Micro-computed tomography (micro-CT) has potential for three-dimensional reconstruction of detailed tooth structure and has been used to analyze sophisticated morphology of teeth in experimental animals such as mice and rats [2–5]. The study we conducted was designed to investigate root development quantitatively using micro-CT and periodontal tissue formation qualitatively in allogenic tooth transplantation between rat littermates [6].

2 Micro-CT Analysis and Histology for Allogenic Tooth Transplantation

The upper right second molar germs in 2-week-old rats were extracted and immediately transplanted into the upper right first molar socket of rat littermates under anesthesia. The upper left second molars in 2-week-old recipient rats were used as a control. The rats were fixed and tissues analyzed at 0, 4, 8 or 12 weeks after transplantation. Root elongation of seven rats in each group was analyzed quantitatively with micro-CT. Periodontal tissue formation was examined qualitatively with histology.

3 Roots Elongation and Periodontal Tissue Formation After Allogenic Transplantation

Roots elongated after allogenic transplantation, but they were significantly shorter than control roots. The number of roots varied from 1 to 4 in transplanted teeth, while it was consistently 4 in control teeth. Histological examination demonstrated that periodontal tissue formation in transplanted teeth was equivalent to that of the control teeth, whereas about 40% of the transplanted teeth showed inflammation in the dental pulp, about a half of which were accompanied by inflammatory periodontal ligaments.

4 Conclusion

Allogenic transplantation between rat littermates permits root elongation and periodontal tissue formation.

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Booster Effect of Thermal Energy on Bactericidal Action of Hydroxyl Radical Generated by Photolysis of H_2O_2

Midori Shirato, Hiroyo Ikai, Keisuke Nakamura, Eisei Hayashi, Taro Kanno, Keiichi Sasaki, Masahiro Kohno, and Yoshimi Niwano

Abstract. The effect of thermal energy on the yield of and the bactericidal action of hydroxyl radical generated by photolysis of H_2O_2 is discussed in this review article.

Key words. Bactericidal effect, Hydrogen peroxide (H_2O_2), Hydroxyl radical, LED, Thermal energy

1 Introduction

A novel disinfection technique in which fungi and bacteria are killed by hydroxyl radical generated by photolysis of H_2O_2 has been developed in our laboratory [1]. In the disinfection technique, H_2O_2 was irradiated with the visible blue light at wavelength of around 400 nm to generate hydroxyl radical. High concentration of H_2O_2 , however, might cause a kind of adverse effect when the disinfection technique is applied in oral cavity. Hence, low concentration of H_2O_2 is preferable in clinical point of view of safety issues.

Addition of thermal energy to the photolysis of H_2O_2 reaction might decrease the concentration of H_2O_2 with the yield of hydroxyl radical kept unchanged. Regarding

M. Shirato (✉), H. Ikai, E. Hayashi, T. Kanno, and K. Sasaki
Division of Fixed Prosthodontics, Department of Restorative Dentistry, Tohoku University
Graduate School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: shirato@dent.tohoku.ac.jp

K. Nakamura and Y. Niwano
Laboratory for Redox Regulation, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

M. Kohno
New Industry Creation Hatchery Center, Tohoku University, 6-6-10 Aoba,
Aramaki, Aoba-ku, Sendai 980-8579, Japan

the effect of thermal energy on hydroxyl radical generation systems, it was reported that the radical yield increased with water temperature in sonolysis of water [2]. Thus, it is expected that the photolysis of H_2O_2 is also accelerated by temperature rise of H_2O_2 resulting in an increase in the generation of hydroxyl radical.

This review article discusses the influence of the concentration and the temperature of H_2O_2 on the yield of hydroxyl radical its bactericidal activity.

2 Influence of the Concentration of H_2O_2 on Photolysis of H_2O_2

The yield of hydroxyl radical generated by photolysis of H_2O_2 increased with the concentration of H_2O_2 . When 500, 750, and 1,000 mM H_2O_2 were irradiated with the light at 400 nm at 25°C, the yield of hydroxyl radical were 15.5, 21.1, and 24.4 $\mu\text{M}/\text{min}$, respectively.

The bactericidal effect of the disinfection technique based on the photolysis of H_2O_2 at 25°C also depended on the concentration of H_2O_2 . *Streptococcus mutans* was killed with a >5-log reduction when 1,000 mM H_2O_2 was photolyzed for 3 min. On the other hand, when the concentration of H_2O_2 was lowered to 750 mM, a 3 min-treatment resulted in only >4-log reduction of *S. mutans*.

3 Influence of Temperature of H_2O_2 on Photolysis of H_2O_2

Photolysis of H_2O_2 was enhanced by the temperature rise leading to an increase in hydroxyl radical generation as observed in sonolysis of water. The generation rate of hydroxyl radical in photolysis of 500 mM H_2O_2 at 45 and 55°C were 21.6 and 26.5 $\mu\text{M}/\text{min}$, respectively.

The bactericidal effect of the disinfection technique on *S. mutans* was also enhanced dependently on the temperature of H_2O_2 . *S. mutans* was killed with a >5-log reduction by the technique at 45°C in 2 min. Furthermore, a 5-log or more reduction was achieved only in 1 min when the temperature was risen to 55°C. The result clearly revealed that the photolysis of 500 mM H_2O_2 at 55°C could reduce oral pathogenic bacteria more efficiently than the photolysis of 1,000 mM H_2O_2 at 25°C even though the yields of hydroxyl radical are almost the same under the both conditions.

4 Conclusion

It is suggested that the temperature rise enhances synergistically the bactericidal action of photolysis of H_2O_2 , and the temperature effect is more efficient than the increase in H_2O_2 concentration.

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Epigallocatechin Gallate Disturbs Thymidine Glycol Formation Induced by Oxidative Stress in Sjögren's Syndrome-Like Autoimmune Sialadenitis of MRL/lpr Mice

Keiichi Saito, Shiro Mori, Masao Ono, and Takeyoshi Koseki

Abstract. In this study, we administered EGCG to MRL/lpr mice from age of 8–16 weeks for 57 days, and thereafter examined immunohistochemically the expression of thymidine glycol (TG), heme oxygenase-1 (HO-1) and ssDNA in autoimmune sialadenitis of submandibular glands of ten EGCG-administered MRL/lpr mice compared with those of six no EGCG-conspecifics controls using streptavidin-biotin methods. TG is formed in nuclei when ROS oxidizes deoxy thymidine and HO-1, one of antioxidants, is induced by green tea catechin. We found that EGCG-administered MRL/lpr mice showed attenuated expression of TG and ssDNA and enhanced HO-1 immunostaining in salivary gland duct epithelial cells. Furthermore, the significant positive correlation existed between TG and ssDNA expression, and the significant inverse correlation was noticed between TG and HO-1 expression. In conclusion, our results indicate that EGCG ameliorates the detrimental influences of oxidative stress on salivary gland tissues in autoimmune sialadenitis of MRL/lpr mice.

Key words. Epigallocatechin gallate, Sjögren's syndrome, Thymidine glycol

K. Saito (✉) and T. Koseki

Division of Preventive Dentistry, Department of Oral Health
and Development Sciences, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: saitok@m.tohoku.ac.jp

S. Mori

Department of Maxillofacial Surgery, Tohoku University Hospital, Sendai, Japan

M. Ono

Department of Pathology, Tohoku University Graduate School of Medicine, Sendai, Japan

1 Introduction

Reactive oxygen species (ROS) has been shown to be produced by the actions of Th1 cytokines and autoantibodies, and to be causative of Sjören's syndrome development. On the other hand, epigallocatechin gallate (EGCG) contained in green tea possesses antioxidant properties and alleviates ROS-related tissue damages. HO-1 induced by EGCG plays a crucial role in its antioxidant actions. TG is found in nuclei of injured cells by oxidization of deoxy thymidine. Single strand DNA (ssDNA) is expressed in nuclei of apoptotic cells. Thus, the expression of TG or ssDNA suggests that salivary gland tissues are impaired by oxidative stresses.

2 Materials and Methods

We administered EGCG dissolved in drinking water to MRL/lpr mice from age of 8–16 weeks for 57 days, and an average of dosage was 592 µg per a day and a mouse. Specimens of submandibular gland tissues were obtained from ten EGCG-administered MRL/lpr mice and six no EGCG-conspecific control mice. Paraffin-embedded tissue sections (3 µm) were immunostained against TG, HO-1 and ssDNA using streptavidin-biotin methods. The primary antibodies we utilized were a mouse monoclonal antibody anti-TG (clone 2E8) (Japan Institute for the Control of Aging, Shizuoka, Japan), goat polyclonal anti-HO-1 (Santa Cruz Biotechnology, Inc., CA) and rabbit polyclonal anti-ssDNA (DAKO Japan Inc, Kyoto, Japan). The specimens were subjected to wet autoclave treatment in the presence of 0.01M citrate buffer pH6.0 (120°C, 5 min) for antigen retrieval. Immunological controls were incubated without the primary antibodies or with non-immune serums, and showed negligible immunoreactions. We compared the rates of expression of HO-1 and ssDNA between EGCG-provided and TG-negative (EGCG(+)TG(-)) mice and no EGCG control and TG-positive (EGCG(-)TG(+)) mice. Moreover, we assigned four staining level degrees to staining level scores of 0–3 as follows: undetectable=0, weak and negligible=1, moderate=2, intense=3.

3 Results and Discussion

The submandibular gland tissue sections prepared from autoimmune sialadenitis of EGCG(+)MRL/lpr mice showed intense HO-1 expression, and negligible expression of TG and ssDNA in duct epithelial cells. On the other hand, in EGCG(-) control mice, TG- and ssDNA-positive staining were observed, but not HO-1 staining. Additionally, it was represented that the staining level scores of HO-1 were raised, in contrast, those of TG and ssDNA declined significantly in EGCG(+)-populations compared with the corresponding ones of EGCG(-)-controls. The percentage of

HO-1-positive specimens was significantly higher, and those of TG-positive and ssDNA-positive ones were significantly lower in nine EGCG(+)TG(-) mice than those of six EGCG(-)TG(+) mice.

Evaluation of Kendall rank correlation coefficients between TG staining level scores and those of HO-1 and ssDNA revealed that there were significant inverse correlation between TG and HO-1 expression and significant positive correlation between TG and ssDNA expression.

4 Conclusions

Our results indicate that EGCG ameliorates the detrimental influences of oxidative stress on salivary gland tissues in autoimmune sialadenitis of MRL/lpr mice and suppresses salivary gland tissue damages.

The Influence of Mastication on the Dopaminergic System in the Rat Brain

Hiroki Takahashi, Yoshihito Funaki, Akito Tsuboi,
Makoto Watanabe, and Satoshi Yamaguchi

Abstract. There are a few findings between reduced sensory input from mastication and neurochemical denaturation of the brain. The aim of this study was to investigate the effect of different mastication on the dopaminergic system in the rat striatum using brain microdialysis (MD) and positron emission tomography (PET).

Key words. Dopaminergic system, Microdialysis, Positron emission tomography, Rat

1 Introduction

There are several findings between reduced information from masticatory system, such as teeth, periodontal tissues, oral mucosa, tongue and masticatory muscles, and neurochemical denaturation of the brain. The Soft diet is considered to reduce the sensory information from oral cavity and also to modulate the chewing pattern. The aim of this study was to investigate the effects of the soft diet on the dopaminergic system in the rat striatum using brain microdialysis (MD) and positron emission tomography (PET). In addition, we examined the effect of the aging on the dopaminergic system.

H. Takahashi (✉), A. Tsuboi, M. Watanabe, and S. Yamaguchi
Division of Aging and Geriatric Dentistry, Graduate School of Dentistry,
Tohoku University, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: hiroki@mandible.dent.tohoku.ac.jp

Y. Funaki
Cyclotron and Radioisotope Center, Tohoku University, Sendai 980-8578, Japan

2 Materials and Methods

2.1 Animals

Male Fischer 344 rats (3 weeks old, $n=8$) were used in this study. These rats were divided into three groups: S-8 weeks, S-60 weeks and P-60 weeks. Group S-8 weeks rats were fed solid diet reach to 8 weeks old ($n=3$). Group S-60 weeks rats were fed solid diet reach to 60 weeks old ($n=2$), and group P-60 weeks rats were fed powder diet reach to 60 weeks old ($n=3$).

2.2 Post-synaptic Experiment

The amount of DA receptors was measured using PET system (Clairvivo PET, SHIMADZU). Each rat of group-S and -P was anesthetized with ketamine/xylazine (120 mg/kg + 20 mg/kg) and injected (about 20 MBq, i.v.) with ^{11}C -raclopride (^{11}C -RAC) which is an imaging probe for DA- D_2 receptor, and dynamic brain scan was carried out for 60 min. PET image had not information where the radioactivity was present in the rat brain, thus PET-CT fusion images were created to obtain anatomical information.

2.3 Pre-synaptic Experiment

Rats were anesthetized with pentobarbital (50 mg/kg, i.p) and implanted with a guide cannula into the left striatum (coordinates: AP 0.7, L 3.0, V 4.0 mm). After surgical operation, the location of the guide cannula was immediately confirmed by CT. After three days of the implantation, the dialysates were collected by MD system, and the extracellular concentrations of DA were measured by high performance liquid chromatography-electron capture detector.

3 Results

3.1 PET Study

The accumulation of ^{11}C -RAC was observed in the rat striatum. The binding of ^{11}C -RAC in the rat striatum was no significant difference between S-8 weeks and P-60 weeks.

3.2 MD Study

The total extracellular concentrations of DA in 100 min from the striatum of P-60 weeks were increased 1.15 ± 0.63 (mean $\pm$ S.D.) times compared with that of S-8 and -60 weeks. However there was no significant difference between S-8 weeks and S-60 weeks.

4 Conclusion

It is thought that the methods of this study are available to observe the influence of mastication on the neurochemical denaturation of the brain at the molecular level. These findings suggest that the mastication influenced on the DA release at the pre-synapse but not on the amount of DA receptors at post-synapse in the rat striatum.

Functional Micro-organization of the Macaque Postcentral Somatosensory Cortex Representing Orofacial Structures

Takashi Toda and Haruhide Hayashi

Abstract. The somatotopical representation of orofacial structures is well established in the postcentral somatosensory cortex of primates (SI). The inspection of functional micro-organization in SI should lead to understanding of the neural basis that underlies dexterous orofacial functions. There, nearby neurons within a local region sometimes coded anatomically-discrete but functionally-related portions, such as the tongue tip and labial mucosa. Also, nearby pairs of neurons responded to a natural stimulus with varying degrees of temporal correlation. It is likely that a local region of SI is organized in a less redundant manner than previously thought.

Key words. Columnar organization, Neuron, Receptive field, Somatosensory cortex, Temporal correlation

1 Introduction

The notion of modular organization in SI has been widely documented for over 50 years. In this hypothesis, the columnar assembly of the neurons that are 300–600 μm wide (so called “column”) is considered to be a functional unit of information processing [1]. However, the functional significance of the column is still not fully understood. To address this issue, it is essential to compare physiological features between nearby neurons in SI. Here, we will briefly summarize our findings on functional diversity within a local region of SI [2, 3].

T. Toda (✉) and H. Hayashi
Division of Physiology, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: ttoda@m.tohoku.ac.jp

2 Spatial Extent of Nearby Neuron Receptive Fields

We used SI neurons recorded in areas 3b and 1 of conscious monkeys [2]. Briefly, the distance between adjacent two neurons recorded consecutively along the tracks (neuron-pairs) was estimated based on the distance reading of the electrode manipulator during the experiment. In the pairs having interneuronal distances less than 300 μm , we compared the receptive fields (RFs) of the two neurons, omitting neurons that lacked apparent RFs. Consequently, most of the pairs (c.a., 90%) were associated with the same or continuous portions of a single oral structure or continuous portions of neighboring structures. However, the rest (c.a., 10%) were associated with discrete but functionally-related oral portions of different structures. We speculate that the latter type of organization might be suitable neural substrates for detecting objects straddling different orofacial structures.

3 Temporal Variability of Nearby Pyramidal Neuron Activity

We used pairs of SI neurons recorded simultaneously in areas 1 and 2 of one conscious monkey [3]. The pairs were classified into three types: pairs of putative interneurons (I-I pairs), pairs of a putative pyramidal neuron and interneuron (P-I pairs), and pairs of putative pyramidal neurons (P-P pairs). A numerical method was adopted that evaluates the difference of the gross temporal profile over several hundreds of millisecond or more [4]. That is, each discrete spike train was first smoothed by convolving with a Gaussian function. Then, the normalized dot product (similarity index) was taken between a pair of spike trains. The method successfully quantified the gross temporal difference of activity in each pair of SI neurons. The similarity indexes were significantly lower in P-P pairs than in I-I pairs. On the other hand, it is generally accepted that the activities of pyramidal neurons represent the net result of local information processing [5]. Taken together, the temporal diversity of pyramidal neurons activity within a small SI region might help the brain to monitor the time course of sustained tactile stimulation, such as the onset, duration, and offset.

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Morroniside Derivative Regulates E-Selectin Expression in Human Endothelial Cells

Naomi Tanigawa, Yoshinori Takeda, Fortunatus Sunghwa, Masayuki Ninomiya, Makoto Hagiwara, Mamoru Koketsu, and Kenji Matsushita

Abstract. E-selectin is particularly of interest in the case of inflammatory diseases owing to its expression in the activated endothelium. E-selectin mediates cell tethering and rolling interactions through the recognition of sialofucosylated lewis carbohydrates expressed on circulating leukocytes. This phenomenon serves as an important trigger in inflammatory response. We prepared three morroniside derivatives and harpagoside as a positive control and then examined the effects of these compounds on E-selectin expression in human endothelial cell cultures. We found that 7-*O*-cinnamoylmorroniside significantly suppressed the expression of E-selectin induced with TNF- α ($IC_{50}=49.3 \mu M$). Furthermore, it was more active than another cinnamic-acid-conjugated iridoid glycoside (harpagoside;

N. Tanigawa and K. Matsushita (✉)

Department of Oral Disease Research, National Institute,
National Center for Geriatrics and Gerontology, Obu 474-8511, Japan
and

Department of Geriatric Oral Science, Tohoku University Graduate School of Dentistry,
4-1, Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: ntani@ncgg.go.jp; kmatsu30@ncgg.go.jp

Y. Takeda

Department of Chemistry, Faculty of Engineering, Gifu University,
1-1 Yanagido, Gifu 501-1193, Japan

F. Sunghwa

Department of Chemistry, College of Natural and Applied Sciences,
University of Dar es salaam, Kinondoni, Tanzania

M. Ninomiya and M. Koketsu

Department of Materials Science and Technology, Faculty of Engineering,
Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

M. Hagiwara

Department of Oral Disease Research, National Institute, National Center
for Geriatrics and Gerontology, Obu 474-8511, Japan

IC_{50} = 88.2 μ M), 7-*O*-methylmorroniside, and morroniside itself. These results suggest that 7-*O*-cinnamoylmorroniside is a potent inhibitor of TNF- α -induced E-selectin expression and that it may be useful as an anti-inflammatory agent.

Key words. Cell-ELISA, E-selectin, Flavonoid

1 Introduction

Flavonoids, such as flavones, flavonols, flavanones, flavanonols, flavans, flavanols, leucoanthocyanidins, anthocyanidins, aurones, chalcones, and isoflavones, are polyphenolic compounds that occur ubiquitously in plants and have a variety of biological effects both in vitro and in vivo. They have been found to have anti-inflammatory activity in both proliferative and exudative phases of inflammation [1].

One of the initial events in inflammation is activation of endothelial cells, which then express cell surface adhesion molecules such as the endothelial leukocyte adhesion molecule (E-selectin) [2, 3]. Inflammatory cytokines such as tumor necrosis factor α (TNF- α) activate endothelial cells to express adhesion molecules and promote synthesis and release of a variety of inflammatory cytokines and chemokines to thereby support recruitment of activated leukocytes to an inflammatory lesion [4].

In this study, we prepared several morroniside derivatives and examined their anti-inflammatory effect by examining E-selectin expression on human endothelial cells in vitro.

2 Materials and Methods

Human umbilical vein endothelial cells (HUVECs) (4×10^4 cells/well) were incubated with 10 ng/mL of TNF- α in the presence and in the absence of test specimens for 2 h. The expression level of E-selectin in HUVECs was measured by an ELISA.

3 Results and Discussion

7-*O*-Cinnamoylmorroniside exhibited excellent anti-inflammatory activity (IC_{50} = 49.3 μ M) by inhibiting the expression of E-selectin, and it was more active than another cinnamic-acid-conjugated iridoid glycoside (harpagoside; IC_{50} = 88.2 μ M), 7-*O*-methylmorroniside, and morroniside itself. As a result, 7-*O*-cinnamoylmorroniside was observed to be a potent inhibitor of TNF- α -induced E-selectin expression.

In this study, we showed that 7-*O*-cinnamoylmorroniside markedly attenuated E-selectin adhesion to TNF- α -activated endothelial cells and that it may inhibit monocyte adhesion on the activated endothelium, thus conferring protection against atherogenic lesion formation. Thus, the compound may hamper initial atherosclerotic events involving endothelial CAM induction.

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Regulation of Osteoblastic Differentiation by the Ubiquitin–Proteasome Pathway

Maki Uyama, Mari Sato, Masamitsu Kawanami, and Masato Tamura

Abstract. In eukaryotic cells, degradation of most intracellular proteins is carried out by the ubiquitin–proteasome pathway. This pathway is the therapeutic target in several diseases. Recent observations suggest that bone metabolism is regulated by the ubiquitin–proteasome pathway, and that proteasome activity is critical for activation of osteogenic transcription factors. The clinical efficacy of bortezomib, a 26S proteasome inhibitor used as an anticancer drug in the treatment of multiple myeloma, has been linked to an increase in bone markers. Also, bortezomib has been reported to induce differentiation of osteoblasts both in vitro and in vivo. From our studies, bortezomib not only induces osteoblastic differentiation but also inhibits myogenic differentiation in pluripotent C2C12 cells in culture.

Key words. Bortezomib, Cell differentiation, Ubiquitin–proteasome pathway

M. Uyama

Biochemistry and Molecular Biology, Graduate School of Dental Medicine,
Hokkaido University, North 13, West 7, Sapporo 060-8586, Japan
and

Periodontology and Endodontology, Graduate School
of Dental Medicine, Hokkaido University, North 13, West 7, Sapporo 060-8586, Japan

M. Sato and M. Tamura (✉)

Biochemistry and Molecular Biology, Graduate School of Dental Medicine,
Hokkaido University, North 13, West 7, Sapporo 060-8586, Japan
e-mail: mtamura@den.hokudai.ac.jp

M. Kawanami

Periodontology and Endodontology, Graduate School of Dental Medicine,
Hokkaido University, North 13, West 7, Sapporo 060-8586, Japan

1 Introduction

The ubiquitin–proteasome pathway plays a pivotal role in the degradation of short-lived and regulatory proteins which is important in the control of basic cellular processes including regulation of differentiation and proliferation. Given its essential role, ubiquitination is a highly regulated process that involves the sequential action of three enzymes termed ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin-protein ligase (E3). The specificity of ubiquitination is often controlled by E3, which facilitates the transfer of ubiquitin to appropriate targets. Poly-ubiquitination of proteins promotes their translocation to the 26S proteasome for degradation. Recent data suggest that the ubiquitin–proteasome pathway is the therapeutic target in several diseases.

Bone mass is dependent on the balance between bone formation by osteoblasts and bone resorption by osteoclasts. The differentiation and function of osteoblasts is regulated by a number of regulatory factors (including bone morphogenetic proteins (BMPs) and Wnt), key signal transduction molecules (such as the Smads and β -catenin), and critical transcription factors (including Runx2 and ATF4). E3 ubiquitin ligases such as Smad-ubiquitination-regulatory factor 1 (Smurf1), beta-transduction repeat-containing protein 1 (β -TrCP1), WW-domain-containing protein 1 (WWP1), and related adapter proteins (e.g., Schnurri-3 (Shn3)) regulate the protein activity of Smads, Runx2 or ATF4 via ubiquitination, thus promoting their proteasome-dependent degradation. Experiments in mice revealed that knocking down Smurf1 results in increased bone mass, while overexpressing Smurf1 decreased bone formation [1]. Shn3 deficient mice also displayed osteosclerosis, increased bone matrix deposition, and augmented osteoblast activity [1]. These observations suggest that bone metabolism is regulated by the ubiquitin–proteasome pathway, and that proteasome activity is critical for the activation of osteogenic transcription factors.

Many structural classes of proteasome inhibitors are known. Among them, certain proteasome inhibitors are bone-anabolic agents in both normal and pathological conditions. Garrett et al. demonstrated that systemic administration of epoxomicin and proteasome inhibitor-1 increased bone volume and bone formation rates in mice [2]. The proteasome inhibitor bortezomib (Velcade™) is used for the treatment of multiple myeloma and mantle cell lymphoma. Multiple myeloma is a plasma cell malignancy characterized by the formation of bone lesions. In these patients, bortezomib exhibits bone-anabolic activity, with increased serum levels of osteogenic markers such as osteocalcin, and increased numbers of osteoblasts in bone biopsies [3]. Recent results from animal studies showed that bortezomib also increased bone mass in normal bone [4].

2 Conclusion

Here we show that the proteasome inhibitor bortezomib, but not lactacystin, epoxomicin, or MG-132, not only induces osteoblastic differentiation but also inhibits myogenic differentiation in pluripotent C2C12 cells in culture. These observations

indicate that the function of the proteasome in controlling degradation of poly-ubiquitinated signaling molecules plays an important role in the decision of cell fate during differentiation in cells such as osteoblasts or myoblasts.

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Downregulation of Dullard Expression Enhances BMP-Dependent Neuritogenesis in PC12 Cells

Ye Zhang, Tada-aki Kudo, Yoshinaka Shimizu,
Mitsuhiro Kano, Yuya Sano, and Hiroyasu Kanetaka

Abstract. The protein phosphatase dullard negatively regulates bone morphogenetic protein (BMP) signaling by promoting the dephosphorylation or degradation of BMP receptors. We investigated the effects of endogenous dullard downregulation on BMP-induced neuritogenesis in a rat PC12 cell line to assess the basic role of dullard in mammalian neuronal differentiation.

Y. Zhang

Department of Physical Medicine and Rehabilitation,
Graduate School of Biomedical Engineering, Tohoku University,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

T. Kudo (✉)

Division of Oral Physiology, Graduate School of Dentistry,
Tohoku University, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: tkudo@m.tohoku.ac.jp

Y. Shimizu

Division of Oral Pathology, Tohoku University Graduate School
of Dentistry, Tohoku University, 4-1 Seiryomachi, Aoba-ku,
Sendai 980-8575, Japan

M. Kano

Division of Oral and Craniofacial Anatomy, Tohoku University Graduate
School of Dentistry, Tohoku University, 4-1 Seiryomachi, Aoba-ku,
Sendai 980-8575, Japan

Y. Sano

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

H. Kanetaka

Department of Physical Medicine and Rehabilitation, Graduate School of Biomedical
Engineering, Tohoku University, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
and

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

Key words. Dullard, Neuronal differentiation, PC12, Phosphatase

1 Introduction

Bone morphogenetic proteins (BMPs) regulate multiple biological processes, including cellular proliferation, differentiation, and early development. Regulation of the BMP signaling pathway is essential for neuronal differentiation. Rat PC12 cells are a model of neuronal differentiation and differentiate into neuron-like cells by the action of BMPs [1]. Cellular protein phosphorylation, modulated by protein kinases and phosphatases, regulates a series of biological functions. The dullard protein phosphatase was initially isolated as a neural region-specific gene from a cDNA library of the lateral regions of *Xenopus* [2]. It contains 244 amino acids with a DXDX(T/V) catalytic motif in a C-terminal phosphatase domain [2, 3], and is an inhibitory factor of BMP signaling. It specifically inhibits BMP signaling by promoting the ubiquitin-mediated proteosomal degradation of BMP receptors [2].

2 Materials and Methods

To assess the role of dullard in PC12 neuronal differentiation, the effects of endogenous dullard downregulation on BMP2-mediated neuronal differentiation were evaluated by conducting image analysis with phase-contrast microscopy, real-time PCR analysis, and Western blotting. Dullard expression was downregulated by electroporation of dullard specific short interfering RNA (siRNA) into PC12 cells.

3 Results

The real-time PCR results showed that endogenous dullard mRNA expression was extensively lowered by RNA interference (RNAi) in PC12 cells and that these cells showed longer neurite outgrowth in the course of BMP-mediated neuronal differentiation; in addition, a higher percentage of PC12 cells than control cells were like neurons. The activity of the downstream BMP signaling pathways (the p38 and Smad signaling pathways) was examined in the knocked-down cells by Western blotting, and the phosphorylation level of Smad1/5/8 tended to be more elevated than that of control cells, whereas the p38 phosphorylation level was not obviously enhanced compared to that of control cells.

4 Conclusion

The present study suggests that dullard functions as an important regulator of BMP-dependent neuronal differentiation in rat PC12 cells. The promotion of neuronal differentiation by dullard downregulation in PC12 cells may have occurred through the Smad signaling pathway but not through the p38 signaling pathway.

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Session II
Host–Parasite Interface

Inhibition of T-Cell-Mediated and Infection-Induced Periodontal Bone Resorption by TACE Blockade

Hiroyuki Kanzaki, Xiaozhe Han, Yukiko Asami, Maiko Suzuki, Toshihisa Kawai, and Martin Taubman

Abstract. Host immune responses trigger promotion of bone resorption in periodontitis, and infiltrated lymphocytes express abundant RANKL, the essential factor for osteoclastogenesis. Since RANKL is initially produced as a membrane-bound protein, cell-to-cell contact can be required for osteoclastogenesis. However, distance between lymphocytes and active osteoclasts is generally observed. This observation presume released cytokine(s) from activated lymphocytes might induce osteoclastogenesis in periodontitis.

Previously, we reported that cleavage of RANKL and TNF- α by TNF- α converting enzyme (TACE) from lymphocytes can play a role in human periodontitis. Herein, we further examined function of TACE in two experimental periodontitis models in rats: (1) *Aggregatibacter actinomycetemcomitans*-reactive T-cell adoptive transfer and (2) *Porphyromonas gingivalis* infection. Anti-TACE antibody was injected into the gingivae to probe the role of TACE in bone destruction. Our data suggested that TACE blockade could inhibit alveolar bone destruction. TACE might be a potentially therapeutic target for periodontitis amelioration.

Key words. Osteoclastogenesis, Periodontitis, sRANKL, TACE, TNF- α

1 Introduction

Host immune responses play a prominent role in promoting bone resorption in periodontitis [1], and lymphocytes found in periodontal lesions express abundant RANKL [2]. RANKL, the essential factor for osteoclastogenesis, is initially produced as a

H. Kanzaki, X. Han, Y. Asami, M. Suzuki, T. Kawai, and M. Taubman (✉)
Department of Immunology, The Forsyth Institute, 245 First Street,
Cambridge, MA 02142, USA
e-mail: mtaubman@forsyth.org

membrane-bound protein and cell-to-cell contact is required for osteoclastogenesis in that physiological condition [3]. However, distance between infiltrated lymphocytes and active osteoclasts on the alveolar bone surface in inflamed periodontal tissue is generally observed [4]. Membrane-bound RANKL can be cleaved by enzymes into sRANKL which maintains biological activity [5]. Therefore, cell-to-cell contact between activated lymphocytes and osteoclasts may not be absolutely necessary for osteoclastogenesis, but cytokine(s) released from activated lymphocytes might also induce osteoclastogenesis in periodontitis.

2 TACE Plays a Role in Osteoclastogenic Cytokine Release from Lymphocytes

Previously, we discovered that cleavage of RANKL by TNF- α converting enzyme (TACE) from lymphocytes plays a role in human periodontitis [6]. TACE can cleave, not only RANKL, but also TNF- α , possibly an alternative osteoclastogenic cytokine [7]. Interestingly, increased TNF- α has been reported in periodontitis [8].

We further examined function of TACE in two experimental periodontitis models in rats: (1) *Aggregatibacter actinomycetemcomitans*-reactive T-cell adoptive transfer and (2) *Porphyromonas gingivalis* infection. Robust TACE expression was observed in both models. sRANKL and TNF- α release into gingival tissue were also increased, and these increases were inhibited by TACE blockade. Alveolar bone resorption was also significantly ameliorated by TACE blockade in both models.

Since synergy between sRANKL and TNF- α on osteoclastogenesis has been reported [9], blockade of release of one or both of these two osteoclastogenic cytokines could effectively inhibit cytokine-dependent osteoclastogenesis.

3 Conclusion

sRANKL and TNF- α release by TACE can play a pivotal role in alveolar bone destruction. TACE might be a potentially therapeutic target for periodontitis amelioration.

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Subgingival Plaque Biofilm Microflora of Elderly Subjects

Yuki Abiko, Takuichi Sato, Reiko Sakashita, and Nobuhiro Takahashi

Abstract. This study aimed to quantify *Porphyromonas gingivalis* (*Pg*) in subgingival plaque of periodontitis (P) and periodontally healthy (H) subjects in the elderly. Subsequently, *fimA* genotypes were determined, and compared between P and H sites in the same subjects. The proportion of *Pg* was higher in P than in H. The *fimA* genotypes of P were identified mainly as II and Ib, while those of H were as I–IV. In four out of five subjects in whom *Pg* was detected in H sites, the *fimA* genotype was identical between P and H sites, but the proportion of *Pg* was significantly higher in P sites than in H sites ($P < 0.05$). This study suggests that the increase of *Pg* and the specific *fimA* genotypes are associated with P in the elderly, and that *Pg* possibly transmits within the oral cavity.

Key words. Biofilm, *fimA*, Microflora, *Porphyromonas gingivalis*, The elderly

Y. Abiko

Division of Oral Ecology and Biochemistry, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
and

Department of Microbiology, School of Dentistry, Aichi-Gakuin University,
Nagoya 464-8650, Japan

T. Sato (✉) and N. Takahashi

Division of Oral Ecology and Biochemistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: tak@m.tohoku.ac.jp

R. Sakashita

Nursing Foundation, College of Nursing Art and Science,
University of Hyogo, Akashi 673-8588, Japan

1 Introduction

The frequency of periodontitis over 65 years is increasing in Japan. This study aimed to quantify *Porphyromonas gingivalis* (*Pg*) in subgingival plaque of periodontitis (P) and periodontally healthy (H) subjects in the elderly. Subsequently *fimA*, a gene encoding virulence-associated fimbriae of *Pg*, were determined, and compared between P and H sites in the same subjects.

2 Quantitative Analysis of *Pg* and Its *fimA* Genotyping

Informed consent was obtained from independent elderly subjects (>60 years), and their subgingival plaque were collected. Total bacteria and *Pg* were quantified by real-time PCR, as reported previously [1]. *fimA* genes of *Pg* were classified into six genotypes (types I, Ib, II–V) by DNA sequence analysis, as described elsewhere [2]. According to the deepest probing depths, subjects were divided into H (<4 mm) and P (≥4 mm) subjects. The proportion of *Pg* was higher in P than in H. The *fimA* genotypes of P were identified mainly as II and Ib, while those of H were as I, II, III and IV. In four out of five subjects in whom *Pg* was detected in H sites, the *fimA* genotype was identical between P and H sites, but the proportion of *Pg* was significantly higher in P sites than in H sites ($P < 0.05$). This study suggests that the increase of *Pg* and the specific *fimA* genotypes are associated with P in the elderly, as well as in P patients aged <60, and that *Pg* possibly transmits within the oral cavity.

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Divalent Cations Enhance Short-Time Fluoride Exposure-Induced Inhibition on Acid Production by *Streptococcus mutans*

Hitomi Domon-Tawaraya, Kazuko Nakajo, Jumpei Washio,
Satoshi Fukumoto, and Nobuhiro Takahashi

Abstract. This study aimed to evaluate the effects of divalent cations on the short-time fluoride exposure induced-inhibition of the pH fall ability of *Streptococcus mutans*. In the presence of divalent cations, short-time fluoride exposure enhanced the inhibition of pH fall by *S. mutans*, probably due to the fluoride bound to bacterial cell via cations. Thus, it is suggested that the pre-rinse with divalent cations increases fluoride retention to plaque bacteria and subsequently enhances fluoride inhibition on acid production by dental plaque in vivo.

Key words. Divalent cations, Fluoride, Inhibition of acid production, Short-time effect, *Streptococcus mutans*

1 Introduction

Daily mouthrinse with a low concentration of fluoride is effective for caries prevention. This caries prevention is caused by stimulating remineralization of tooth surface and by inhibition of caries-related bacterial acid production [1, 2]. Furthermore,

H. Domon-Tawaraya

Division of Pediatric Dentistry, Tohoku University Graduate School
of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
and

Division of Oral Ecology and Biochemistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

K. Nakajo, J. Washio, and N. Takahashi (✉)

Division of Oral Ecology and Biochemistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: nobu-t@dent.tohoku.ac.jp

S. Fukumoto

Division of Pediatric Dentistry, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

it has been demonstrated that divalent cations such as Ca^{2+} and Mg^{2+} increase the fluoride-binding to bacterial cells [3]. However, the effect of a short-time exposure of fluoride on oral bacteria, mimicking fluoride rinsing in the oral cavity, in the presence of divalent cations is unclear. Thus, we aimed to examine effects of divalent cations on the short-time fluoride exposure-induced inhibition of one of the most acidogenic and caries-related oral bacteria, *Streptococcus mutans* in vitro, in an environment which simulates the oral cavity.

2 Short-Time Fluoride Exposure Inhibits pH Fall by *Streptococcus mutans* in the Presence of Divalent Cations

Short-time fluoride exposure (250–950 ppm) inhibited acid-production by *S. mutans* in the presence of divalent cations even after washing twice prior to glucose addition, while no inhibitory effect on acid production in the absence of divalent cations. In addition, the pH fall inhibition increased with the amount of fluoride bound to bacterial cells. These results suggest that the inhibitory effect is attributed to the bacteria-bound fluoride, which is released from bacterial cells as environmental pH is acidified through bacterial glucose fermentation. The released fluoride ion may turn to hydrogen fluoride (HF) efficiently at acidic pH and subsequently penetrate the cell membrane of bacteria in acidic environment [4], resulting in inhibition of the glycolytic enzymes of *S. mutans* including enolase [5].

3 Supply of Divalent Cations as Pre-rinse for a Short-Time Topical Fluoride Application

This study revealed that divalent cations enhance the short-time fluoride exposure induced-inhibition on acid production of *S. mutans*, probably because fluoride can bind to bacterial cells efficiency via divalent cations. Therefore, it is expected that a short-time topical fluoride application after pre-rinse with divalent cations can stimulate the binding of fluoride to plaque bacteria, even after the washing with saliva, drinking and eating. The enhanced binding of fluoride to dental plaque might reduce the risk of caries in terms of inhibitory effect of fluoride on acid production by plaque bacteria.

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Silent Aspiration of Oral Bacteria in Elderly Patients

Ayako Hasegawa, Takuichi Sato, Yasushi Hoshikawa,
Takashi Kondo, and Nobuhiro Takahashi

Abstract. This study aimed to quantify and identify bacteria existing in intraoperative bronchial fluids (IBF), and evaluate the relationship between cough and/or swallowing reflexes (CSR) impairment and silent aspiration of oral bacteria (SAOB) in elderly patients. The bacterial amounts in IBF of patients with impaired CSR were higher than those with normal CSR. Predominant isolates from IBF with normal CSR were *Streptococcus*, *Porphyromonas*, *Olsenella*, *Gemella* and *Veillonella*, while those with impaired CSR were *Streptococcus*, *Veillonella*, *Eikenella* and *Prevotella*. These results indicate that IBF contains bacteria, probably derived from the oral microbiota, and suggest that SAOB occurs in elderly patients irrespective of impairment of CSR.

Key words. 16S rRNA, Bronchial fluids, Oral bacteria, Silent aspiration, The elderly

1 Introduction

Postoperative pneumonia (POP) may occur when cough and/or swallowing reflexes (CSR) are impaired, and thus silent aspiration of oral bacteria (SAOB) is possibly involved in POP. This study aimed to quantify and identify bacteria existing in intraoperative bronchial fluids (IBF), and evaluate the relationship between CSR impairment and SAOB in elderly patients.

A. Hasegawa, T. Sato (✉), and N. Takahashi
Division of Oral Ecology and Biochemistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: tak@m.tohoku.ac.jp

Y. Hoshikawa and T. Kondo
Department of Thoracic Surgery, Institute of Development, Aging,
and Cancer, Tohoku University, Sendai 980-8575, Japan

2 Measurements of CSR, Anaerobic Culture and Bacterial Identification

Informed consent was obtained from patients with pulmonary carcinoma (mean age, 72.4 ± 6.8 years). Prior to the lung resections, cough reflex was measured using a nebulised citric acid delivered by an ultrasonic nebuliser, while swallowing reflex was measured by a bolus injection of distilled water into the pharynx through a nasal catheter. IBFs from patients undergoing lung resections were collected with the micro-sampling probe, and were cultured anaerobically on blood agar plates. After 7 days, colony forming units were counted and isolated bacteria were identified by 16S rRNA gene sequencing, as described previously [1–3].

The bacterial amounts in IBF of patients with impaired CSR were higher than those with normal CSR, although the differences were not statistically significant. Predominant isolates from IBF with normal CSR were *Streptococcus*, *Porphyromonas*, *Olsenella*, *Gemella* and *Veillonella*, while those with impaired CSR were *Streptococcus*, *Veillonella*, *Eikenella* and *Prevotella*. These results indicate that IBF contains bacteria, probably derived from the oral microbiota, and suggest that SAOB occurs in elderly patients irrespective of impairment of CSR.

Acknowledgment This study was supported in part by Grants-in-Aid for Scientific Research (20591661, 22390399, 23592791, 23659866) from the JSPS.

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Priming Effect of Vitamin D₃ Analog on Human Monocytic Cells in Response to Microbe-Related Ligands, Especially NOD2 Agonistic Muramyl dipeptide

Tomoko Ikeuchi, Takashi Nakamura, Satoshi Fukumoto,
and Haruhiko Takada

Abstract. Activated vitamin D₃, 1 α ,25-dihydroxyvitamin D₃, exhibits various biological functions, including immunomodulating activities. Human monocytic THP-1 cells were primed with a vitamin D₃ analog, OCT, followed by stimulation with various Toll-like receptor (TLR), NOD1 and NOD2 ligands. The primed cells produced higher IL-8 than non-primed cells upon stimulation with the ligands. NOD2-agonistic muramyl dipeptide (MDP) was the highest inducer of IL-8 production in the primed cells, and IL-8 production increased until 72 h-priming with OCT. OCT up-regulated the expression of NOD2 and the primed cells exhibited higher activation of p38, JNK and ERK in the MAPK pathway, I κ B α in the NF- κ B pathway, and TAK1 upstream in both pathways than non-primed cells. Analysis using inhibitors indicated that activation of the above signal molecules is required for the priming activity.

Key words. Innate immunity, MAPK, NF- κ B, NOD2, Vitamin D₃

Vitamin D is a mainplayer in calcium autoregulation and exhibits immunomodulating effects. We examined possible regulation of innate immune responses by vitamin D₃ analog, 1 α ,25-dihydroxy-22-oxavitamin D₃ (OCT), and found priming activity of

T. Ikeuchi

Division of Oral Microbiology, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
and

Division of Pediatric Dentistry, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

T. Nakamura and S. Fukumoto

Division of Pediatric Dentistry, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

H. Takada (✉)

Division of Oral Microbiology, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: dent-ht@m.tains.tohoku.ac.jp

OCT on human monocytic THP-1 cells to enhance production of IL-8 in response to various microbe-related synthetic TLR, NOD1 and NOD2 ligands. Therefore, we investigated mechanisms of the priming activity, with special reference to NOD2-agonistic MDP.

We demonstrated up-regulation of expression of NOD2 mRNA in OCT-treated THP-1 cells in terms of RT- and real-time-PCR and flowcytometry, respectively. Then, we examined possible enhanced activation of signal molecules in the OCT-treated cells upon stimulation with MDP. First, we examined TAK1, because TAK1 is a downstream molecule in the NOD2 signaling, and is an upstream both in the MAPK signaling pathway and I κ B α activation prior to NF- κ B activation. Expectedly, THP-1 cells incubated with 5Z-7-oxozeaenol, a TAK1 inhibitor, exhibited clear reduction of IL-8 production in response to MDP stimulation in Western blot analysis, whereas in the presence of 5Z-zeaenol, an inactive control of TAK1 inhibitor, IL-8 production by THP-1 cells in response to MDP increased markedly. Next, we examined possible involvement of MAPK signaling pathway; ERK, p38, and JNK. OCT-primed cells exhibited enhanced phosphorylation of the molecules in response to MDP stimulation as compared with non-primed cells. Both U1026 and SB203580 as inhibitors of ERK and p38, respectively, reduced IL-8 production by THP-1 cells in response to MDP stimulation. Finally, we demonstrated that OCT-primed cells exhibited enhanced phosphorylation of I κ B α in response to MDP stimulation as compared with non-primed cells. Wedelolactone, an IKK inhibitor, reduced IL-8 production in a dose-dependent manner by THP-1 cells in response to MDP stimulation.

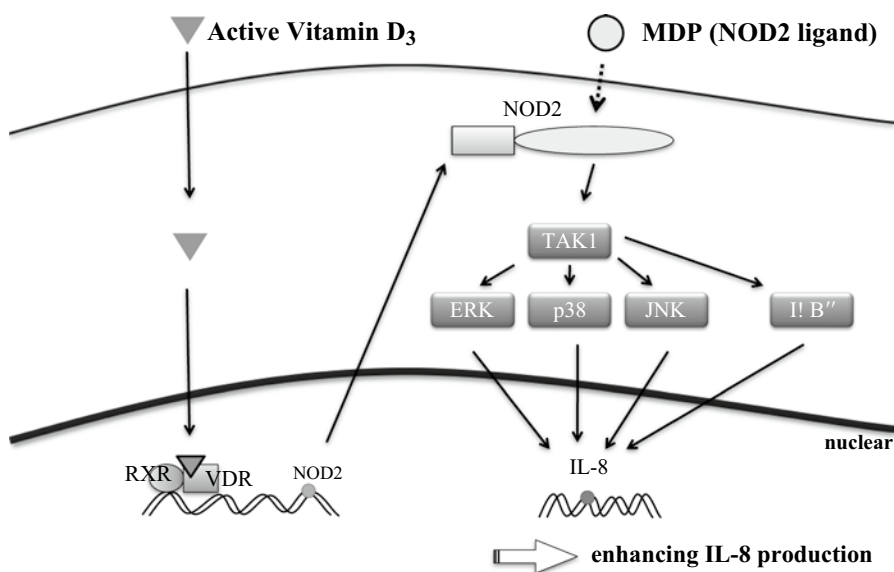


Fig. 1. Possible mechanism of the priming activity of vitamin D₃ to enhance innate immune response to NOD2-agonistic MDP

Taking together the above findings, it was revealed that the priming activity of the vitamin D₃ up-regulated NOD2 expression and augmented activation of TAK1, MAPK and NF-κB pathways are involved in enhanced IL-8 production by OCT-primed human monocytic cells in response to MDP (see Fig. 1). It is reasonable to assume the induction of various innate immune responses by activated vitamin D₃.

A Method for Quantitatively Evaluating Plaque Biofilm-Removing Capacity of a Dental Water Jet Using EPMA

Kazuo Kato, Kiyomi Tamura, Haruo Nakagaki, Shoichi Sakakibara, Youki Ou, Susumu Matsumoto, Kana Fujita, and Takuichi Sato

Abstract. Inconsistent results have been reported on the effects of dental water jet (DWJ) on plaque removal. Plaque biofilm removing capacity of the DWJ was evaluated by a new method to measure biofilm thickness using an electron-probe micro-analyzer (EPMA). Area measurements of biofilm cross-sections imaged with backscattered electrons suggested that irrigation using a DWJ would be an effective means of plaque control.

Key words. Backscattered electron image, Biofilm removing capacity, Oral irrigator, Quantitative evaluation

1 Introduction

The DWJ has been used as an adjunct device to tooth-brushing to enhance plaque control. It is known that oral irrigation with a pulsating jet stream of water generates benefits for gingival health, such as the reduction of bleeding and gingivitis, the improvement of periodontal pockets, and a decrease in periodontal pathogens. However, these studies were inconsistent in the effects of the DWJ on plaque removal, reporting both positive and negative results.

K. Kato (✉), K. Tamura, and H. Nakagaki
Department of Preventive Dentistry and Dental Public Health, School of Dentistry,
Aichi-Gakuin University, 1-100 Kusumoto-cho, Chikusa-ku, Nagoya 464-8650, Japan
e-mail: kazkato@dpc.aichi-gakuin.ac.jp

S. Sakakibara, Y. Ou, S. Matsumoto, and K. Fujita
Department of Pediatric Dentistry, School of Dentistry, Aichi-Gakuin University,
2-11, Suemori-dori, Chikusa-ku, Nagoya 464-8651, Japan

T. Sato
Department of Oral Biology, Tohoku University Graduate School of Dentistry,
4-1, Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

2 Plaque Biofilm Formation and Water Irrigation

Seven consenting subjects (19–24 years) wore in situ plaque-generating devices [1], which comprised a pair of enamel slabs attached to the upper molars for 2 days. Each device, upon removal from the mouth, was clamped, and one of the slab surfaces was treated with the DWJ (Dentrex, Ricoh Elemex Corporation), irrigated at pressure settings of 707 kPa for 5 s at a distance of 3 mm from the surface. Another slab with no treatment served as control.

3 Electron Microscopy

The biofilms on the slabs were freeze-dried and sputter-coated with platinum to examine their surfaces using EPMA for secondary-electron imaging. The biofilm slab units were then embedded in methacrylate and cross-sectioned in the center to examine using backscattered electron compositional (COMPO) imaging. A thin layer of platinum that coincided with the outer surface of the biofilm could easily be identified by atomic number contrast through the COMPO images (Fig. 1), which are used to detect contrast between areas with different chemical compositions.

4 Evaluation of Biofilm Removal

The area between the enamel and the outer biofilm surface, indicated by thin platinum layer, was measured in the COMPO images to calculate the average thickness of the biofilm on the specimen. The biofilm-removal capacity through irrigation could be estimated using the reduced rate of biofilm thickness, demonstrating that

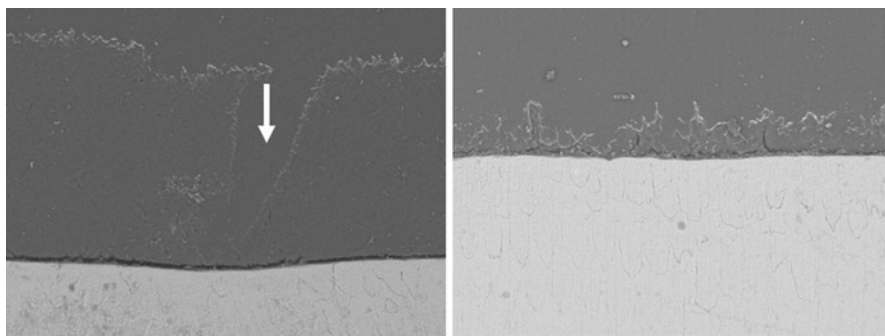


Fig. 1. COMPO images ($\times 1,000$) of the cross-sectioned slabs with treatment (*right*) and without treatment (*left*). An arrow indicates the fissure caused by shrinkage of the biofilm

approximately 90% of plaque biofilm can be removed by vertical irrigation with water. It would be worthwhile to investigate further the properties of plaque biofilms treated with a water jet device, such as the rate of regeneration or the penetration of pharmaceutical agents into the tissue, in order to apply the device successfully as an adjunct for plaque control.

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Bicarbonate Decreases Inhibitory Effects of Fluoride on Acid Production by *Actinomyces naeslundii*

Junko Kawashima, Kazuko Nakajo, Jumpei Washio, Hidetoshi Shimauchi, and Nobuhiro Takahashi

Abstract. This study aimed to evaluate the effects of bicarbonate and fluoride on the acid production ability of *Actinomyces naeslundii*. In the presence of bicarbonate, the inhibitory effect of fluoride was decreased, suggesting that *Actinomyces* species have a unique mechanism to escape or weaken the inhibitory effect of fluoride. This might be relevant to that *Actinomyces* species has a bicarbonate-assimilating pathway for succinate production in addition to the glycolytic pathway.

Key words. Acid production, *Actinomyces naeslundii*, Bicarbonate, Fluoride, mutans streptococci

1 Introduction

Currently in Japan, among the people aged 60 or over, the prevalence of root-surface caries was reported about 50% [1] and is predicted to increase in step with the aging of the population in the future. *Actinomyces* species, a saccharolytic and

J. Kawashima (✉)

Division of Periodontology and Endodontology, Tohoku University
Graduate School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
and

Division of Oral Ecology and Biochemistry, Tohoku University
Graduate School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: kawashima@dent.tohoku.ac.jp

K. Nakajo, J. Washio, and N. Takahashi

Division of Oral Ecology and Biochemistry, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

H. Shimauchi

Division of Periodontology and Endodontology, Tohoku University Graduate
School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

acidogenic bacterium, has been isolated in a high proportion from root-surface caries (ca. 13%) as well as oral streptococci (ca. 30%) [2], suggesting the association of this bacterium with root-surface caries. It is known that bicarbonate contained in saliva stimulates the growth [3] and the acid production [4] of *Actinomyces*, while fluoride, a preventive agent for dental caries, inhibits the acid production of *Actinomyces* and the inhibition is more effective at acidic environment [5]. However, effects of coexistence of bicarbonate and fluoride, as in the oral cavity, on the acid production are still unclear. Thus, we aimed to examine effects of bicarbonate and fluoride on the acid production of the representative *Actinomyces* species, *Actinomyces naeslundii* in vitro.

2 Bicarbonate Enhanced the Fluoride-Tolerance of Acid Production by *A. naeslundii*

In the presence of bicarbonate, the inhibition by fluoride of the acid production of *A. naeslundii* was lower than in the absence of bicarbonate. The inhibition was higher at acidic pH than at neutral pH, while the inhibition was lower in the presence of bicarbonate again. These results clearly indicate that bicarbonate enhanced the fluoride-tolerance of acid production from sugar by *A. naeslundii*. The higher inhibition by fluoride at acidic pH could be attributed to the fact that fluoride ions may turn to hydrogen fluoride (HF) and subsequently penetrate the cell membrane of bacteria at acidic pH [6]. The intracellular fluoride ions may inhibit efficiently the glycolytic enzymes, including enolase [7].

3 The Fluoride-Tolerance of *Actinomyces naeslundii* Was Higher than mutans streptococci

When compared with mutans streptococci, which was reported in our previous study [8], the acid production of *A. naeslundii* appears to be more tolerant to fluoride, suggesting that *Actinomyces* species have a unique mechanism to escape or weaken the inhibitory effect of fluoride. It is known that *Actinomyces* possesses a bicarbonate-assimilating pathway for succinate production in addition to the glycolytic pathway [9], while mutans streptococci, as well as other oral streptococci, utilize the glycolytic pathway mainly for acid production. This difference in metabolic pathway might be relevant to the fluoride tolerance of this species.

The fluoride tolerance of *Actinomyces* species, as well as its enhancement by bicarbonate, should be considered for topical fluoride application for the prevention of root-surface caries.

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A New Method to Evaluate pH at the Interface Between Parasites and Biomaterials

Gen Mayanagi, Koei Igarashi, Jumpei Washio, Kazuko Nakajo,
Hitomi Domon-Tawaraya, and Nobuhiro Takahashi

Abstract. Physiochemical assessment of the parasite–biomaterial interface is essential to develop new biomaterials. The purpose of this study was to develop a method to evaluate pH at the parasite–biomaterial interface and demonstrate physiochemical interaction at the interface. An experimental apparatus with a well for pH monitoring was made of acrylic resin with a bottom of test materials. The well was filled with *Streptococcus mutans* NCTC 10449, and an ion-sensitive field-effect transistor (ISFET) pH electrode was placed at an interface between bacterial cells and materials. The pH was monitored after the addition of 1% glucose at 37°C. The pH at the parasite–biomaterial interface was successfully monitored using the experimental apparatus with an ISFET pH electrode. This method could be useful to assess the pH at the parasite–biomaterial interface.

Key words. Biomaterial, Ion-sensitive field-effect transistor pH electrode, Parasite, pH, Physiochemical interaction

G. Mayanagi (✉)

Research Unit for Interface Oral Health Science, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: genm@dent.tohoku.ac.jp

K. Igarashi, J. Washio, K. Nakajo, and N. Takahashi

Division of Oral Ecology and Biochemistry, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

H. Domon-Tawaraya

Division of Oral Ecology and Biochemistry, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
and

Division of Pediatric Dentistry, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

1 Introduction

Tooth-restoration interface provides a niche for bacteria to inhabit and the risk of secondary caries may increase. Therefore, biomaterials for restoring the teeth are desired to regulate bacterial activity, such as bacteriostatic properties, and thus a method to assess these properties of biomaterials is essentially needed.

An ion-sensitive field-effect transistor (ISFET) pH electrode is very small, highly sensitive due to low internal electric resistance [1]. Thus, the ISFET pH electrode enabled us to measure *in vivo* acid production by human dental plaque, that is, pH at the interface between tooth surface and dental plaque [2].

The purpose of this study was to develop a new apparatus to assess pH at the interface between parasites and biomaterials *in vitro*, using the ISFET pH electrode, and to evaluate the inhibitory effect of materials on bacterial acid production from sugar at the bacteria–material interface.

2 Experimental Apparatus and Measurement of pH

An experimental apparatus with a well (4.0-mm diameter and 2.0-mm deep) for pH monitoring was made of acrylic resin with a specimen of biomaterials at the bottom. An ISFET pH electrode (model PH-60 T1; Nihon Kodon, Tokyo, Japan) was placed on the materials. Cells of *Streptococcus mutans* NCTC 10449 were packed into the well and kept at 37°C for 10 min. Then, 500 μ L of 1% glucose was added onto the cells. The pH was monitored continuously with a pH meter (ISFET mV/pH METER; BAS, Tokyo, Japan) attached to a chart recorder (LR 4220; Yokogawa Electric Corporation, Tokyo, Japan) in an incubator (Merck KGaA, Darmstadt, Germany) at 37°C for 90 min.

3 Physiochemical Interaction at the Interface Between *S. mutans* and Biomaterials

In this apparatus, the pH was stable for about 10–20 min after glucose addition, because it took time for glucose to diffuse into *S. mutans* cells and also for *S. mutans* to produce acids from glucose. When the acids penetrated the interface between *S. mutans* and biomaterials, the pH started to decrease. On the other hand, the material components were expected to diffuse into the interface gradually, affecting bacterial sugar metabolism and organic acid production. In addition, bacterial acidification probably influenced the diffusion of material components from the corresponding material. The pH at the interface can be determined due to these interactions between bacteria and biomaterial. Using this experimental apparatus with an ISFET pH electrode, the pH at the bacteria–dental cement interface was successfully monitored [3].

4 Conclusion

This new method is useful to assess the pH at the parasite–biomaterial interface. The advantages of this method are that the pH at the parasite–biomaterial interface can be measured directly, considering physiochemical interactions between bacteria and biomaterials. This method will be able to evaluate the bacteriostatic activity of biomaterials and will also contribute to the development of new biomaterials for caries prevention.

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Acid Tolerance and Endogenous Acid Production by Oral bifidobacteria

Kazuko Nakajo, David Beighton, and Nobuhiro Takahashi

Abstract. This study focused on the responses of oral bifidobacteria to acidification and their ability of endogenous acid production without an extracellular energy source. In the absence of exogenous glucose, the viability and the stability of intracellular pH of bifidobacteria were maintained at acidic pH, besides bifidobacteria continued to produce acid even at acidic pH. The continuous acid production from endogenous energy source in acidic environment may contribute to acid tolerance of bifidobacteria in the absence of extracellular energy source.

Key words. bifidobacteria, Endogenous acid production, mutans streptococci, pH_{in}, Viability

1 Introduction

Although bifidobacteria are members of the microflora in the human gastro-intestinal tract, they also inhabit in the human oral cavity. Among oral bifidobacteria, *Bifidobacterium dentium* and *Bifidobacterium longum* are known to be isolated together with mutans streptococci from acidic caries lesions [1]. Adaptation and tolerance to acidification are important factors for the survival of bifidobacteria in the acidic environment in the gastro-intestinal tract and caries lesions. It is well known that the cell-membrane-bound, proton-translocating ATPase (H⁺-ATPase) is an important enzyme for maintaining the intracellular pH (pH_{in}) by excreting protons

K. Nakajo (✉) and N. Takahashi

Division of Oral Ecology and Biochemistry, Tohoku University Graduate School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: nakajo@m.tohoku.ac.jp

D. Beighton

Dental Institute, King's College London, Floor 17, Guy's Tower,
St. Thomas Street, London SE1 9RT, UK

from the cell of several bacteria, including some species bifidobacteria under acidic conditions [2, 3]. In caries lesions, energy sources such as carbohydrate are not always available, and bacteria living there have to survive in an acidic environment without carbohydrate supply. Thus we focused on the viability and the pH_{in} maintenance ability under acidic conditions and the endogenous acid production of *B. dentium* and *B. longum* as compared with those of representative mutans streptococci, *Streptococcus mutans*, in the absence of an extracellular energy source.

2 Acid-Tolerance of Bifidobacteria Was Higher than mutans streptococci in the Acidic Environment Without Extracellular Energy Source

B. dentium and *B. longum* kept a higher viability after acidification for 3 h at pH 4.0, when compared with *S. mutans*. Furthermore, at a pH of <5.0, the pH_{in} maintenance ability of both bifidobacterial species was higher than that of *S. mutans*. These results indicate that both bifidobacterial species were found to be more acid-durable and more capable to maintain pH_{in} than *S. mutans* in the acidic environment without extracellular energy source, glucose. The high viability and pH_{in} maintenance ability of bifidobacteria in the acidic environments may explain why bifidobacteria exist as stable species in acidic caries lesions, together with mutans streptococci. However, the mechanism of the high pH_{in} maintenance ability in the absence of extracellular energy source was not elucidated at that stage when we reported these results [4].

3 The Activity of Endogenous Acid-Production by bifidobacteria Was Higher than mutans streptococci

B. dentium and *B. longum* exhibited the activity of pH fall as well as *S. mutans* in the presence of extracellular sugar, glucose, while in the absence of glucose, the activity of pH fall of both bifidobacterial species was higher than that of *S. mutans*. Particularly, *B. longum* continued to produce acid from endogenous energy source even at pH <4.5. The acid production from endogenous energy source of bifidobacteria implies that these bacteria possess the metabolic pathways specific for glycogen synthesis and degradation, as reported previously in streptococci [5], in which glycogen is synthesized through the formation of ADP-glucose and degraded by phosphorolysis. The store of endogenous energy source and the continuous acid-production in acidic environment may contribute to acid tolerance of bifidobacteria in the absence of extracellular energy source, since the endogenous energy production can maintain pH_{in} appropriately against extracellular acidification through the activity of H⁺-ATPase. In addition, the acid production without extracellular energy source may promote cariogenic potential in oral bifidobacteria in vivo.

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Maternal Transmission of Mutans and Other Oral Streptococcal Species

Asami Otake-Asakawa, Rikako Harada-Oikawa, Yuko Ohara-Nemoto, Mitsuro Tanaka, and Shigenobu Kimura

Abstract. In this study, the distribution of mutans and non-mutans streptococci in the plaque samples from 320 children was investigated, and then the maternal transmission in the 16 mother-and-child pairs was assessed. In the children's plaque, 14 oral streptococci including mutans streptococci (MS) were identified, although the frequencies of the species were quite different among species. The relatively higher number of species was detected in the MS-positive children than the MS-negative one. In the mother-and-child pairs, the genotypic fingerprinting analysis using pulse-field gel electrophoresis (PFGE) revealed a high frequency in matching genotypes of MS. Furthermore, the distribution as well as the quantitative level of non-mutans streptococci was similar to each other. Thus, the present results strongly suggested that the maternal transmission does occur in not only mutans streptococci but also non-mutans streptococci.

Key words. Maternal transmission, Mutans streptococci, Non-mutans streptococci, Pulse-field gel electrophoresis

A. Otake-Asakawa (✉), R. Harada-Oikawa, and M. Tanaka
Division of Pediatric Dentistry, Iwate Medical University School of Dentistry,
1-3-27 Chuodori, Morioka, Iwate 020-8505, Japan
e-mail: aotake@iwate-med.ac.jp

Y. Ohara-Nemoto
Department of Oral Molecular Biology, Nagasaki University
Graduate School of Biomedical Sciences, Nagasaki, Japan

S. Kimura
Division of Molecular Microbiology, Iwate Medical University School of Dentistry,
Iwate, Japan

1 Introduction

Streptococcus mutans and *S. sobrinus* are clearly key causative organisms in human dental caries, and their early acquisition in childhood leads to a high frequency of dental caries in children. However, it was also reported that the composition of non-mutans streptococcal microflora could be a factor that governs the cariogenic potential of MS, suggesting that non-mutans streptococci can contribute to, and modulate, the strength of the cariogenic challenge at a site. However, the maternal transmission of non-mutans streptococci remains to be elucidated. In this study, the distribution of MS and non-mutans streptococci in children's plaques, and then the maternal transmission in mother-and-child pairs were assessed.

2 Methods

2.1 Subjects

The 320 children and 16 mother-and-child pairs with informed consent were participated in this study [approved by the Ethics Committees of IMUSD (D-01023, D-01072)].

2.2 Identification of Oral Streptococci

The streptococci were identified by the culture method with biochemical tests (streptogram[®]), and the seven oral streptococci were identified by the species-specific PCR assays.

2.3 PFGE of MS

The genotypic fingerprinting analysis by PFGE was performed on the *Sma* I-derived fragments from the isolates of *S. mutans* and *S. sobrinus*.

3 Results and Conclusion

The 14 oral streptococci including MS were identified in the children's plaque. Among them, *S. mutans* was most frequently detected, and *S. sobrinus* was scarcely colonized. In the mother-and-child pairs, the PFGE analysis revealed a high

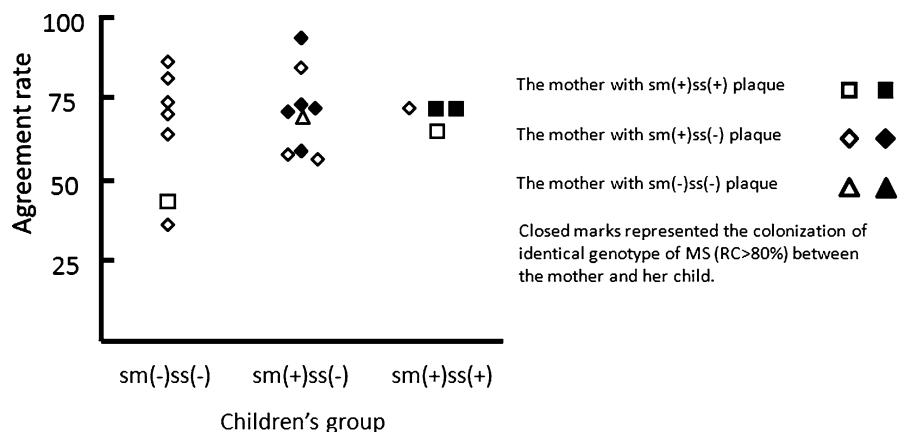


Fig. 1. The agreement rate of the oral streptococcal colonization in the MS-positive and MS-negative children and maternal transmission of MS assessed by PFGE. sm, *S. mutans*; ss, *S. sobrinus*

frequency in matching genotypes of both MS. Furthermore, the subject-based analysis indicated that the detection patterns of non-mutans streptococci as well as the quantitative levels of each bacterial species were quite similar to each other (Fig. 1). Taken together, the present results strongly suggested that the maternal transmission does occur in not only mutans streptococci but also non-mutans streptococci.

A Highly Sensitive alamarBlue® Method for Evaluating Bacterial Adhesion to Biomaterials

Yoko Sakuma, Jumpei Washio, Yasuhisa Takeuchi, Keiichi Sasaki,
and Nobuhiro Takahashi

Abstract. It is known that biofilm formed on the denture surface (denture plaque) causes various oral diseases and the aspiration pneumonia. For prevention of these diseases, new denture materials that inhibit or reduce the formation of denture plaque are strongly required. In order to develop these materials, it is essential to evaluate the bacterial adhesion to material surfaces. Thus, we established a new non-radioisotopic method for evaluating the adhesion of bacteria, using alamarBlue®, a fluorescent reduction–oxidation indicator, that responds quantitatively to the bacterial metabolic activity. Furthermore, we enhanced the sensitivity of the method by adding bacterial metabolic substrates to the reaction mixture. It is suggested that the simple and precise quantification of bacterial adhesion to material surfaces becomes possible by this improved alamarBlue® method.

Key words. Acrylic resin, alamarBlue®, Bacterial adhesion, Biomaterials, Denture plaque

Y. Sakuma (✉) and Y. Takeuchi

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate School of Dentistry, 4-1, Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
and

Division of Oral Ecology and Biochemistry, Tohoku University Graduate School of Dentistry, 4-1, Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: sakuma@dent.tohoku.ac.jp

J. Washio and N. Takahashi

Division of Oral Ecology and Biochemistry, Tohoku University Graduate School of Dentistry, 4-1, Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

K. Sasaki

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate School of Dentistry, 4-1, Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

1 Introduction

It is suggested that biofilm formed on the denture surface (denture plaque) causes various oral diseases such as periodontitis, caries [1, 2] and stomatitis [3], and furthermore, relates to the aspiration pneumonia [4]. The control of the denture plaque is crucial for the prevention of these diseases. Therefore, new denture materials that can inhibit or reduce bacterial adhesion and the formation of denture plaque are strongly required. In order to develop these denture materials, it is essential to establish a quantitative method that evaluates bacterial adhesion to material surfaces.

2 From the Conventional Radioisotopic Method to the New Non-radioisotopic Method

Since the amount of bacteria adhered to the surface of biomaterials is very small, it was difficult to evaluate the amount of adhered bacteria. Thus, the method using radioisotope (RI)-labeled bacteria was usually used for the evaluation. However, this method requires an exclusive-use facility and a strict safety regulation [5].

Recently, the alamarBlue® method have been developed as a non-radioisotopic quantitative method [6]. alamarBlue® is a fluorescent dye that changes fluorescence intensity by oxidation–reduction reaction, a bacterial metabolic reaction [7]. The number of bacterium is able to be calculated from the amount of bacterial metabolic activity, namely the change of fluorescence intensity.

We applied this method to evaluate the adhesion of representative oral bacteria (*Streptococcus mutans*, *Streptococcus sanguinis*, *Actinomyces naeslundii*, *Actinomyces oris* and *Veillonella atypica*) to the surface of the acrylic resin. However, the sensitivity was not enough to quantify the bacterial adhesion.

3 The Enhancement of the Sensitivity of alamarBlue® Method by the Addition of Bacterial Metabolic Substrates

The sensitivity of the alamarBlue® method was enhanced by adding bacterial metabolic substrates (glucose or sodium lactate) to the reaction mixture. The addition of the substrates increased fluorescence intensity of the alamarBlue® in all the bacteria tested and subsequently shortened the reaction time to obtain a fluorescence intensity enough to quantify the bacterial adhesion.

It is suggested that the simple and precise quantification of bacterial adhesion to material surfaces becomes possible by this improved alamarBlue® method.

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Fibronectin Binding Activity of *Streptococcus anginosus* Promotes Adherence to Mucosal Epithelial Cells

Minoru Sasaki, Yoshitoyo Kodama, Yu Shimoyama, Taichi Ishikawa, and Shigenobu Kimura

Abstract. *Streptococcus anginosus* inhabit dental plaque, and its infection to oral mucosa could be associated with oral squamous cell carcinoma. However, the adhesive mechanism to oral mucosa remained to be elucidated. In this study, the adhesive abilities of *S. anginosus* to mucosal epithelial cells and extracellular matrix (ECM) proteins were investigated. The results indicated that *S. anginosus* can adhere to the mucosal epithelial cells (HEp-2 cells) as other oral streptococci do, and that the adhesive ability could be ascribable to mainly its fibronectin-mediated adherence. Furthermore, the adhesive ability to HEp-2 cells of the *S. anginosus* isolates from oral cancer tissues was significantly higher than that of the isolates from the plaque sample of healthy subjects. Thus, the present findings indicate that the fibronectin-mediated adherence could play an important role in the initial process of *S. anginosus* infection to oral mucosa.

Key words. Adherence, Fibronectin binding activity, Oral mucosal epithelial cells, *Streptococcus anginosus*

1 Introduction

Although *Streptococcus anginosus* is a part of the normal flora found in human dental plaque, recent studies indicate that *S. anginosus* infection in oral mucosa can be associated with oral squamous cell carcinoma. The organism possesses a number of pathogenic properties, however, the adhesive mechanism that mediates the initial

M. Sasaki (✉), Y. Kodama, Y. Shimoyama, T. Ishikawa, and S. Kimura
Division of Molecular Microbiology, Department of Microbiology,
Iwate Medical University School of Dentistry, 2-1-1 Nishitokuta, Yahaba-cho,
Shiwagun, Iwate 028-3694, Japan
e-mail: msasaki@iwate-med.ac.jp

process of *S. anginosus* infection to oral mucosal epithelial cells remains to be elucidated. In this study, the adhesive abilities of *S. anginosus* to mucosal epithelial cells of a human larynx carcinoma cell line (HEp-2 cells) as well as the immobilized ECM proteins including fibronectin, laminin and collagen were investigated.

2 Materials and Methods

S. anginosus NCTC 10713 and the clinical isolates were used. The adhesive ability of *S. anginosus* was assessed by the binding of [^3H]-labeled bacteria to the cultured HEp-2 cells and the immobilized ECM proteins.

3 Results and Conclusion

S. anginosus can adhere to HEp-2 cells as other oral streptococci do. *S. anginosus* also showed a significant adhesive ability to the immobilized fibronectin but not to laminin. Furthermore, the adhesive ability to HEp-2 cells as well as the immobilized fibronectin, of the *S. anginosus* isolates from oral cancer tissues was significantly higher than that of the isolates from the plaque sample of healthy subjects (Fig. 1). Taken together, the present findings indicate that the fibronectin-mediated adherence could play an important role in the initial process of *S. anginosus* infection to oral mucosa, leading to the onset of oral squamous cell carcinoma.

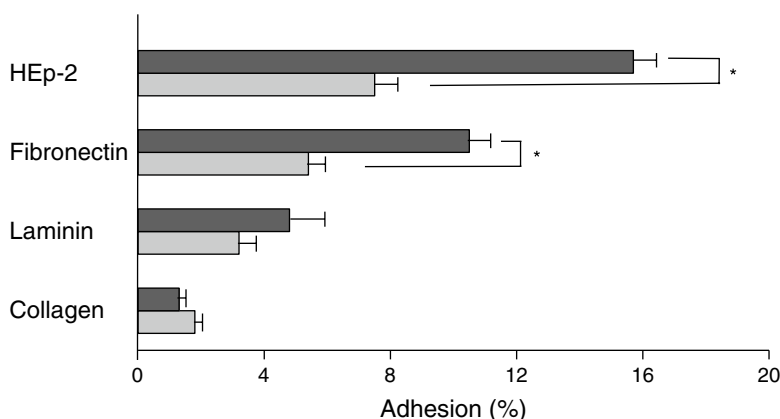


Fig. 1. Adhesion of *S. anginosus* to HEp-2 cells and ECM proteins. Results for *S. anginosus* isolated from the oral cancer tissue of cancer patients (black rectangle) and the isolates from the dental plaque of the healthy volunteer (grey rectangle), are expressed relative to the total binding.

*, $p < 0.05$

Rapid Identification of *Abiotrophia/Granulicatella* Species by 16S rRNA-Based PCR and RFLP

Yu Shimoyama, Minoru Sasaki, Yuko Ohara-Nemoto,
Takayuki K. Nemoto, Taichi Ishikawa, and Shigenobu Kimura

Abstract. *Abiotrophia/Granulicatella* species formerly known as nutritionally variant oral streptococci now consist of five species, *Abiotrophia defectiva*, *Granulicatella adiacens*, *Granulicatella elegans*, *Granulicatella para-adiacens* and *Granulicatella balaenopterae*. In this study, a rapid and highly sensitive identification method for the five *Abiotrophia/Granulicatella* species was developed. The developed method combines the species-specific PCR assays targeting 16S rRNA genes and the 16S rRNA gene PCR amplification followed by a restriction fragment length polymorphism analysis (PCR-RFLP). The species-specific PCR assays enabled to detect exclusively *A. defectiva*, *G. elegans* and *G. balaenopterae* with high sensitivities of 5 pg of genomic DNA, and showed high specificities. However, *G. adiacens* and *G. para-adiacens* cannot be distinguishable each other by a simple PCR assay. Then, the PCR-RFLP with *BsmI* was developed for the detection of *G. adiacens* and *G. para-adiacens*, which also yielded highly sensitive and specific identification of these two species.

Key words. *Abiotrophia/Granulicatella*, Identification, Restriction fragment length polymorphism assay, Species-specific PCR assay

Y. Shimoyama (✉), M. Sasaki, T. Ishikawa, and S. Kimura
Division of Molecular Microbiology, Iwate Medical University, School of Dentistry,
2-1-1 Nishitokuta, Yahaba-cho, Shiwa-gun, Iwate 028-3694, Japan
e-mail: yushimo@iwate-med.ac.jp

Y. Ohara-Nemoto and T.K. Nemoto
Department of Oral Molecular Biology, Nagasaki University Graduate
School of Biomedical Sciences, Nagasaki, Japan

1 Introduction

Abiotrophia/Granulicatella species are one of the major pathogens of infective endocarditis, accounting for 5–6% of the occurrences. However, the identification has been rather hard and sometimes inconclusive by conventional culture methods since these bacteria are nutritionally variant and thus fastidious and slow growing. In this study, we developed a rapid and highly sensitive identification method for the five *Abiotrophia/Granulicatella* species by means of the species-specific PCR assays and the 16S rRNA gene PCR amplification followed by a restriction fragment length polymorphism analysis (PCR-RFLP).

2 Materials and Methods

The species-specific PCR assays targeting 16S rRNA genes for *Abiotrophia defecativa*, *Granulicatella elegans* and *Granulicatella balaenopterae* were developed in this study. The PCR assay was essentially performed as previously described [1]. For the specific identification of *Granulicatella adiacens* and *Granulicatella para-adiacens*, *G. adiacens*/*G. para-adiacens*-specific PCR was developed, and then the PCR-RFLP with *BsmI* was performed.

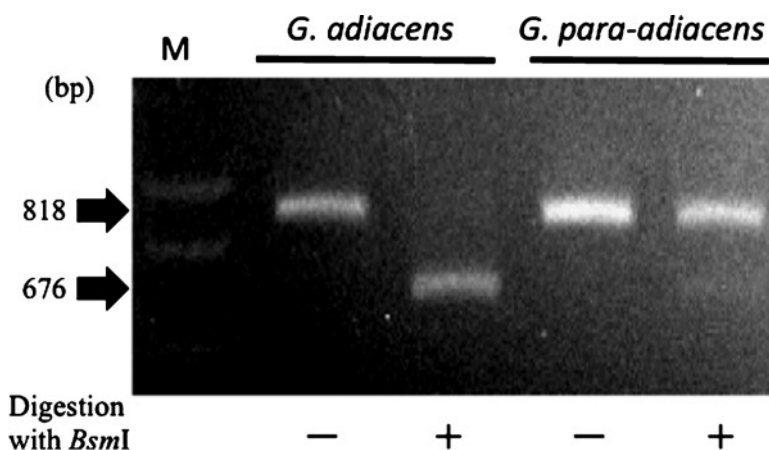


Fig. 1. PCR-RFLP with *BsmI* for specific identification of *G. adiacens* and *G. para-adiacens*

3 Results and Discussion

The developed species-specific PCR assays enabled to detect exclusively *A. defectiva*, *G. elegans* and *G. balaenopterae* with high sensitivities of 5 pg of genomic DNA, corresponding to 100 CFU of bacteria. The specificities were confirmed using other 16 oral bacterial species including 11 oral streptococci, *Streptococcus pyogenes*, 2 staphylococci, *Escherichia coli* and *Porphyromonas gingivalis*. Since *G. adiacens* and *G. para-adiacens* cannot be distinguishable each other by a simple PCR assay, the PCR-RFLP with *BsmI* was developed. The PCR-RFLP also yielded highly sensitive and specific identification of these two species (Fig. 1).

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Quantification and Identification of Bacteria in Maxillary Obturator-prostheses

Yasuhisa Takeuchi, Kazuko Nakajo, Takuichi Sato, Yoko Sakuma, Shigeto Koyama, Keiichi Sasaki, and Nobuhiro Takahashi

Abstract. The aims of this study were to quantify and identify bacteria in the in-use acrylic resin maxillary obturator-prostheses. Aspiration pneumonia-associated or airborne bacteria such as *Klebsiella* and *Pseudomonas* species as well as oral bacteria were detected at $5.0 \times 10 - 2.5 \times 10^6$ colony forming units (CFU)/mL. This study revealed that several species of viable bacteria are present within the obturators. It is suggested that the inner space of obturator can act as bacterial reservoirs.

Key words. Acrylic resin, Bacterial detection, Bacterial identification, Maxillary obturator-prostheses

Y. Takeuchi (✉) and S. Koyama

Clinical Division of Maxillofacial Prosthetics, Tohoku University Hospital,
Sendai 980-8574, Japan
e-mail: takeuchi0620@dent.tohoku.ac.jp

K. Nakajo, T. Sato, and N. Takahashi

Division of Oral Ecology and Biochemistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

Y. Sakuma

Division of Oral Ecology and Biochemistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
and

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate
School of Dentistry, Sendai 980-8575, Japan

K. Sasaki

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate
School of Dentistry, Sendai 980-8575, Japan

1 Introduction

Acrylic resin maxillary obturator-prostheses are required to close the acquired defects, resulting from neoplasm or injuries, and to separate between oral and nasal cavities of the post-maxillectomy patients. When the defect is large, the inside of the obturator is hollowed to reduce the weight of prostheses. It has been estimated that approximately 500–700 species of microorganisms inhabit the surface of teeth, gingival sulcus, tongue and oral mucosa [1], as well as the surface of acrylic resin maxillary obturator-prostheses. Furthermore, saliva and nasal secretion sometimes accumulate in the inner space of acrylic resin obturators (inner-fluid) [2], and thus microorganisms also possibly penetrate into the obturators. It has been reported the existence of microorganisms in the inner space of silicone-based obturators [3], but those in the inner space of acrylic resin obturators are still unclear. Therefore, the aims of this study were to quantify and identify the bacteria in the inner space of acrylic resin obturators. When there was no inner-fluid, 1.0 mL of sterile buffer solution was injected to the inner space of obturator and then collected the solution as a sample.

2 Viable Bacteria Inhabit within Maxillary Obturator-prostheses

Viable bacteria were detected within in-use maxillary obturator-prostheses at 5.0×10^4 – 2.5×10^6 CFU/mL. The densities of bacteria in the inner-fluid samples were higher than those in the no inner-fluid samples, indicating that the inner-fluids support the bacterial growth in obturators.

Bacterial species in the obturators was less diverse than those of plaque and saliva, suggesting that the environment of the inner space of obturator allows limited bacterial species to grow. Only *Lactobacillus* species, known as oral bacteria, were isolated from the no inner-fluid samples. While from the inner-fluid samples, *Klebsiella* and *Pseudomonas* species were predominantly isolated, which are known as aspiration pneumonia-associated or airborne bacteria [4]. These findings suggest that the bacteria in the inner space of obturators were derived from oral, nasal and respiratory regions as well as from the airborne contaminants during fabrication or repair of obturators [5].

3 Clinical Implication

The present study revealed that viable bacteria are present within in-use acrylic resin obturator-prostheses. It is suggested that the acrylic resin maxillary obturator-prostheses, both their surfaces and internal areas, can act as microbial reservoirs. The bacteria and their metabolites in the inner space of acrylic resin obturators may cause the oral and respiratory infections in individuals wearing obturator-prostheses.

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Breath Acetone in Type 1 and Type 2 Diabetes Mellitus

Naoko Tanda, Yoshinori Hinokio, Jumpei Washio, Nobuhiro Takahashi,
and Takeyoshi Koseki

Abstract. Difference of breath acetone between type 1 and type 2 diabetes mellitus (DM) is examined under informed consent. We measure acetone in mouth air and in deep breath by Gas Analyzer® XG-100V (New Cosmos Electric Co., Ltd., Osaka, Japan) and volatile sulfide compound in mouth air by Breathtron® (New Cosmos Electric Co., Ltd., Osaka, Japan), after meal. The acetone ratio of deep exhaled breath to mouth air and concentration of volatile sulfide compound (VSC) are compared between type 1 and type 2 DM patients. The acetone ratio of deep exhaled breath to mouth air is significantly higher in type 1 DM than type 2 DM patients. The concentration of VSC shows no significant difference. Though more cases should be studied, there is a possibility that the acetone ratio might reflect metabolic disorder of type 1 DM patients.

Key words. Acetone, Type 1 diabetes mellitus, Type 2 diabetes mellitus

N. Tanda (✉) and T. Koseki

Division of Preventive Dentistry, Department of Oral Health
and Development Sciences, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: ntanda@m.tohoku.ac.jp

Y. Hinokio

Department of Diabetes and Metabolism, Sendai City Hospital,
3-1 Shimizukouji, Wakabayashi-ku, Sendai 984-8501, Japan

J. Washio and N. Takahashi

Division of Oral Ecology and Biochemistry, Department of Oral Biology,
Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

1 Introduction

Exhaled volatile organic compounds (VOCs) represent ideal biomarkers of endogenous metabolism and could be used to noninvasively measure circulating variables, including human glucose [1]. The sweet smell of acetone in breath accompanies uncontrolled diabetes, a fishy smell is a result of liver disease and a urine-like smell is related to kidney failure [2, 3]. Acetone is a three-carbon ketone body derived from acetoacetate through spontaneous decarboxylation or enzymatic conversion (via acetoacetate decarboxylase) [4]. Acetone can be detected in breath because of its volatile nature. As breath acetone increases during diabetic ketoacidosis [5], fasting, and high-fat diets [6, 7], it is a reliable indicator of circulating ketone bodies [5, 8]. There are, however, few reports about acetone in deep exhaled air and mouth air in DM patients.

2 Acetone in Exhaled Air and Mouth Air

We measured acetone in mouth air and in deep breath by Gas Analyzer[®] XG-100V (New Cosmos Electric Co., Ltd., Osaka, Japan) and volatile sulfide compound in mouth air by Breathtron[®] (New Cosmos Electric Co., Ltd., Osaka, Japan), after meal. The acetone ratio of deep exhaled breath to mouth air and concentration of volatile sulfide compound (VSC) were compared between type 1 and type 2 DM patients. The acetone ratio of deep exhaled breath to mouth air was significantly higher in type 1 DM than type 2 DM patients. The concentration of VSC showed no significant difference.

3 Conclusion

Though more cases should be studied, there is a possibility that the acetone ratio might reflect metabolic disorder of type 1 DM patients.

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Combination of Mutanase and Dextranase Effectively Suppressed Formation of Insoluble Glucan Biofilm by Cariogenic Streptococci

Hideaki Tsumori, Atsunari Shimamura, Yutaka Sakurai,
and Kazuo Yamakami

Abstract. Cariogenic streptococci produce α -1,3-glucan and α -1,6-glucan, which are the major components of dental biofilms. To investigate the possibility of preventing dental biofilm formation, utility of a combination of mutanase and dextranase was examined in vitro. Experiments: Insoluble glucan biofilms were synthesized by cariogenic streptococci (*Streptococcus mutans* 10449, *S. mutans* Ingbritt, *Streptococcus sobrinus* 6715, and *S. sobrinus* B13) in the presence of mutanase and/or dextranase in 96-well microplates. Mutanase from *Paenibacillus humicus* inhibited glucan biofilm formation by these streptococci. This inhibitory effect by mutanase was enhanced in the presence of dextranase. We investigated the effect of combination of these glucosidases on the synthesis of α -1,3-glucan polysaccharides. The combination of recombinant mutanase and dextranase efficiently inhibited the synthesis of α -1,3-glucan by recombinant glucosyltransferase I on dextran-coated microplates. Conclusion: Mutanase and dextranase should be used in combination as preventive agents to inhibit dental biofilm formation.

Key words. Biofilm, Caries, Dextranase, Mutanase, Streptococci

H. Tsumori (✉) and A. Shimamura
Department of Chemistry, National Defense Medical College,
Namiki, Tokorozawa 359-8513, Japan
e-mail: htsumori@ndmc.ac.jp

Y. Sakurai and K. Yamakami
Department of Preventive Medicine and Public Health, National Defense Medical College,
Namiki, Tokorozawa 359-8513, Japan

1 Introduction

Dental caries represents a significant public health problem in many countries. Cariogenic streptococci form glucan biofilms by attaching to the acquired dental pellicle. These biofilms are mainly composed of α -1,3-glucans (mutan) and α -1,6-glucans (dextran), which are produced by glucosyltransferases of streptococci. The insolubility of glucan biofilms is considered to result from their high mutan contents. Therefore, suppression of mutan formation is important for the prevention of dental caries [1]. Mutanase degrades mutan to low-molecular-weight α -1,3-glucan oligosaccharides with the greater solubility [2]. Previously, mutanase has been isolated from *Paenibacillus humicus* [1]. However, the enzyme is not yet developed as a preventive agent [3]. Dextranase has been used in treatments to reduce biofilm formation. In this study, we observed that the combination of mutanase and dextranase exhibited sufficient inhibitory effects on glucan biofilm formation by cariogenic streptococci.

2 Combination of Mutanase and Dextranase Sufficiently Inhibited the Synthesis of Insoluble Glucan Biofilms

Insoluble glucan biofilms were synthesized by cariogenic streptococci (*Streptococcus mutans* 10449, *S. mutans* Ingbritt, *Streptococcus sobrinus* 6715, and *S. sobrinus* B13) in 96-well microplates in the presence of mutanase and/or dextranase. Mutanase from *P. humicus* (1.0–10 munits/mL) was capable of inhibiting in vitro synthesis of insoluble glucan biofilms by cariogenic streptococci (20–33% inhibition compared to control without the addition of enzyme). Dextranase (1.0 munits/mL) alone partially suppressed glucan film formation (4–8% inhibition), and the digested films lost their ability to adhere to the bottom of the microplates. The combination of mutanase (1.0 munits/mL) and dextranase (1.0 munits/mL) exhibited enhanced inhibitory activity on glucan film formation compared with mutanase or dextranase alone (38–64% inhibition).

3 Combination of Recombinant Mutanase and Dextranase Efficiently Suppressed Mutan Biofilm Formation by Recombinant Glucosyltransferase I

Recombinant glucosyltransferase I, sucrose, recombinant mutanase, and/or dextranase were added to dextran-coated 96-well microplates. On the formation of mutan on biotin-dextran-coated streptavidin microplates, recombinant mutanase inhibited the synthesis of mutan biofilms (62% inhibition compared to control without the enzyme). Dextranase alone partially suppressed mutan film formation (14% inhibition).

The combination of mutanase and dextranase inhibited the synthesis of mutan biofilms (92% inhibition).

Thus, a combination of mutanase and dextranase is the key for inhibiting dental biofilm formation and should be used as a preventive medicine for reducing the amount of insoluble glucan biofilms on tooth surfaces.

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Metabolomics of Oral Plaque Biofilm Using CE-TOFMS

Jumpei Washio, Gen Mayanagi, and Nobuhiro Takahashi

Abstract. The various metabolites produced by oral plaque biofilm directly relate to oral diseases such as dental caries and periodontitis. However, the metabolic properties in oral plaque biofilm have not been clarified because the comprehensive identification and quantification of metabolites, metabolomics in short, was technically difficult. Recently, CE-TOFMS, a combination of capillary electrophoresis and time-of-flight mass spectrometry, has been developed to perform metabolomics, and therefore, we applied CE-TOFMS to metabolomics of oral plaque biofilm.

Consequently, it was suggested that it is possible to detect and quantify metabolites related to the Embden–Meyerhof–Parnas pathway, the pentose-phosphate pathway and the citric-acid cycle, the metabolites related to amino acid metabolisms and amino acids in a small amount of oral plaque biofilm, and that these metabolic pathways function in oral plaque biofilm. The metabolomics research using CE-TOFMS may contribute to the clarification of the etiology of oral plaque biofilm-related diseases.

Key words. Bacteria, CE-TOFMS, Metabolism, Metabolomics, Oral plaque biofilm

J. Washio (✉) and N. Takahashi

Division of Oral Ecology and Biochemistry, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: j-washio@dent.tohoku.ac.jp

G. Mayanagi

Research Unit for Interface Oral Health Science, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

1 Introduction

It is thought that the various metabolites, intracellular metabolic intermediates and end-products produced by metabolic activities of oral plaque biofilm directly relate to oral diseases such as dental caries and periodontitis.

However, the metabolic properties, including functional metabolic pathways and their metabolic regulations, have not been clarified sufficiently because the comprehensive identification and quantification of metabolites, metabolomics in short, was technically difficult.

2 Past Metabolomic Approach to Bacterial Metabolism

In the 1960s, Minakami et al. [1] succeeded in quantifying the metabolic intermediates of the Embden–Meyerhof–Parnas pathway in human red blood cells by a photometry-coupled enzymatic method using glycolytic enzymes [1]. Since then, this method was applied to the analysis of metabolite profile in the various bacteria. However, the targeted metabolites were limited, because a significant amount of sample was required to analyze only one metabolite, thus, it was difficult to measure various metabolites simultaneously in the same sample. In addition, it was difficult to analyze metabolites except for in the Embden–Meyerhof–Parnas pathway, because of unavailability of enzymes analyze metabolites.

3 Metabolomics Using CE-TOFMS

Recently, a new analytical device CE-TOFMS was developed by combination of capillary electrophoresis (CE) and time-of-flight mass spectrometry (TOFMS), which is capable to analyze ionic metabolic intermediates quantitatively and comprehensively even in a very small amount of sample. Thereby, metabolomics research has been growing rapidly in various research fields [2–5].

Therefore, we applied CE-TOFMS to metabolomics of oral plaque biofilm, especially noted the changes in the amount of various metabolites after glucose rinse.

Consequently, it was possible to detect metabolites related to the Embden–Meyerhof–Parnas pathway, the pentose-phosphate pathway, the citric-acid cycle in mg-volume of oral plaque biofilm [6]. Furthermore, amino acids and metabolites related to amino acid metabolism were detected. The metabolite profile of dental plaque biofilm was changed by glucose rinse, suggesting that these metabolic pathways function in oral plaque biofilm in vivo.

CE-TOFMS can monitor directly the metabolic dynamics and regulations in oral plaque biofilm, and the analysis results may contribute to the clarification of the etiology of oral plaque biofilm-related diseases and the development of the effective methods to prevent these diseases.

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Change in Infected Root Canal Microflora During the Course of Root Canal Therapy

Keiko Yamaki, Takuichi Sato, Ayako Hasegawa, Yuki Abiko,
Kazuhiro Hashimoto, Yasuhisa Takeuchi, Junko Matsuyama,
Hidetoshi Shimauchi, and Nobuhiro Takahashi

Abstract. The purpose of the present study was to profile the microflora in infected root canals, using anaerobic culture and molecular biological techniques for bacterial identification, before and after root canal treatment. The mean bacterial count in root canals before treatment was 10^6 , and obligate anaerobes were predominant. The predominant isolates were *Olsenella*, *Pseudoramibacter*, *Propionibacterium* and *Mogibacterium*. On second and third visits, no bacteria were detected in the samples collected after treatment. These results suggest that the environment in root canals is anaerobic and therefore support the growth of anaerobes, and that adequate treatment changes the root canal environment drastically.

Key words. 16S rRNA, Infected root canal, Microbiota, PCR, Root canal therapy

K. Yamaki, K. Hashimoto, and H. Shimauchi
Division of Periodontology and Endodontology, Tohoku University Graduate
School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

T. Sato (✉), A. Hasegawa, Y. Abiko, and N. Takahashi
Division of Oral Ecology and Biochemistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: tak@m.tohoku.ac.jp

Y. Takeuchi
Division of Advanced Prosthetic Dentistry, Tohoku University Graduate
School of Dentistry, Sendai 980-8575, Japan

J. Matsuyama
Division of Pediatric Dentistry, Niigata University Graduate School
of Medical and Dental Sciences, Niigata 951-8514, Japan

1 Introduction

Periapical periodontitis is an infectious and inflammatory disease of the periapical tissues caused by oral bacteria invading the root canal. The purpose of the present study was to profile the microflora in infected root canals, using anaerobic culture and molecular biological techniques for bacterial identification, before and after root canal treatment.

2 Anaerobic Culture and Bacterial Identification

Informed consent was obtained from all subjects. Infected root canals with periapical lesions were included. Samples from infected root canals before and after treatment were collected, followed by anaerobic culture on CDC blood agar plates. After 7 days, colony forming units were counted and isolated bacteria were identified by 16S rRNA gene sequencing, as described previously [1–3]. The bacterial count in root canals before treatment was from 10^2 to 10^7 , and obligate anaerobes were predominant. The predominant isolates were *Olsenella*, *Pseudoramibacter*, *Propionibacterium* and *Mogibacterium*. On second and third visits, no bacteria were detected in the samples collected after treatment, i.e., just before root canal obturation with gutta-percha and sealer. These results suggest that the environment in root canals is anaerobic and therefore support the growth of anaerobes, and that adequate treatment changes the root canal environment drastically.

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Measuring Flow Rates of Saliva for Accurate Diagnosis of Xerostomia

Eiko Yoshida, Jun Suzuki, Emi Ito, Ryoichi Hosokawa, Naoko Tanda, Tohru Tamahara, Jun Harako, Katuhiko Taura, and Takeyoshi Koseki

Abstract. Reduction of flow rate of saliva secretion causes xerostomia, which hardly compromises the quality of life of the patients. Thus, clinical diagnosis of xerostomia should be accurate and practicable to prevent and to strengthen preemptive oral health care in the early stage of xerostomia. There are two type of saliva, resting saliva and stimulated saliva, according to physical status. Because the functions of these two saliva are different, it is needed to measure the flow rates of both saliva for the diagnosis of xerostomia. The flow rate of stimulated saliva was significantly correlated with age, gender, number of sound tooth and body height. Among the healthy subjects, the multiple linear regression analysis indicated that the flow rate of stimulated saliva was correlated with body weight and gender. This indicated that the new criteria of flow rate of saliva would be needed for the therapeutic diagnosis of xerostomia.

Key words. Diagnosis, Flow rate, Resting saliva, Stimulated saliva, Xerostomia

1 Introduction

Oral dryness is one of the common complains among whole generations, regardless their salivary flow rate. Because there are several reasons to feel oral dryness, such as dehydration and mouth-breathing, it is important to distinguish the feeling of dry mouth and hypo-salivation by testing the salivary flow. Reduction of flow rate of saliva secretion causes xerostomia, a sensation of oral dryness, especially elderly

E. Yoshida, J. Suzuki, E. Ito, R. Hosokawa, N. Tanda, T. Tamahara, J. Harako, K. Taura, and T. Koseki (✉)
Division of Preventive Dentistry, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: yobou@dent.tohoku.ac.jp

people who take several medicines for their chronic diseases such as hypertension and depression. Because xerostomia hardly compromises the quality of life (QOL) of the patients, clinical diagnosis of xerostomia should be accurate and practicable to prevent and to strengthen preemptive oral health care against xerostomia. In this paper, we discuss measuring the salivary flow rate for the accurate diagnosis of xerostomia.

2 Measuring Method of the Flow Rate of Saliva

There are two types of saliva according to physical status, resting saliva and stimulated saliva. Resting saliva is secreted to protect oral mucosa and organs at the most period of daily life, while stimulated saliva is secreted in fast flow-rate by stimulation such as mastication. The functions of these two salivae are different, thus, it is needed to measure the flow rates of both salivae for the exact diagnosis of xerostomia.

There are several means of collecting saliva, which are widely used, spitting method, filter paper disc method, and swab method. Spitting method is to ask subject to spit saliva into collecting tube during certain period. Although it is the easiest way among three methods, spitting itself might induce to the secretion of stimulated saliva, and it is hard to measure for the patients complaining dry mouth. Filter paper disc is to set the paper disc at certain location in mouth by operator. It is not suitable for mass screening because the process is complicated. So far in swab method, dentist has to set and remove swab at suitable location in mouth with tweezers. For the mass screening test for xerostomia, we select the spitting method for measuring stimulating saliva and the modified swab test for resting saliva, at the mass examination of general and oral health check-up.

3 Normal Flow Rate of Saliva

From the analysis of stimulated saliva, the flow rate was significantly correlated with age, gender, number of sound teeth and body height. On the other hand, from the analysis of resting saliva, the flow rate was significantly correlated with age and number of sound teeth. These results indicated the protective role of stimulated and resting saliva for supporting oral health with more sound teeth, and the flow rates of both salivae are related with age. It is well-known that the opportunity of administering drugs is increasing according to their age. Eliminating the effects of drug administration, we selected the healthy subjects, who had no systematic diseases and no administrations of daily drugs, from the results of general health check-up. Using these healthy subjects, the multiple linear regression analysis revealed that the flow rate of stimulated saliva was correlated with body weight and gender. This indicated that the new criteria of flow rate of saliva would be needed for the exact diagnosis of xerostomia.

4 Conclusion

To understanding the nature and to apply the therapeutic screening test of xerostomia, measuring flow rates of stimulated and resting saliva would be very important. The diagnostic criteria of the flow rate of stimulated saliva would be needed to concern about the body weight and gender for the accurate diagnosis of xerostomia.

Session III
Biomaterial Interface

Biomechanical Assessment for Fully Edentulous Maxilla with Dental Implants

Takaaki Arahira, Mitsugu Todo, Yasuyuki Matsushita,
and Kiyoshi Koyano

Abstract. In this study, two different types of 3D maxillary bone models varying in the shape and the bone quality were constructed using CT images of a female and a male patient with edentulous maxilla. Implant treatment was then provided to the models using six implants and a prosthesis for each. Finite element analysis was performed to characterize the stress state of maxilla. It is noted that the maximum value in the female model was much higher than that in the male model, mainly owing to the weak structure of maxilla of the female patient.

Key words. Bone quality, Dental implant, Edentulous maxilla, Finite element analysis

1 Introduction

The structure of maxilla is very complicated compared to mandible; for example, hollow structures called maxillary sinus exist in the maxilla, and the bone tissue under this sinus is relatively thin compared to the other bone parts, making implant treatment difficult [1]. Furthermore, age and gender also affect the maxilla structure and therefore, a suitable procedure for implant treatment needs to be established

T. Arahira
Interdisciplinary Graduate School of Engineering and Science, Kyushu University,
6-1 Kasuga-koen, Kasuga 816-8580, Japan

M. Todo (✉)
Research Institute for Applied Mechanics, Kyushu University,
6-1 Kasuga-koen, Kasuga 816-8580, Japan
e-mail: todo@riam.kyushu-u.ac.jp

Y. Matsushita and K. Koyano
Faculty of Dental Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku,
Fukuoka 812-0041, Japan

independently for each of patients with edentulous maxilla. In the present study, two different types of edentulous maxilla models were developed from CT images of a male and a female patients. Implant treatments were then provided to the models. Finite element analysis was performed to understand the strain energy density distribution around implants under a pseudo-occlusion condition and effects of bone structure on the SED distribution were discussed.

2 Materials and Methods

Two different types of 3D maxillary bone models were constructed from CT images. The distribution of the Young's modulus was estimated from the bone mineral density distribution [2, 3]. Six implants were embedded into each of the maxillary models and a metal prosthesis was attached to the tops of the implants. Distributed loads were applied in the tangential direction on the surface of the prosthesis. Finite element analysis was then performed to characterize the strain energy density (SED) distributions around the implants.

3 Results and Discussion

The maximum SED values in the vicinity of the implants are evaluated. It is clearly seen that the maximum SED around the implant 1 was the highest of all the six implants in both models. It should be noted that the SED around the implant 1 in the female model was much higher than in the male model, although the other SED values in the female model were relatively smaller than those in the male model except the implant 2. This clearly indicated that for both the models, the right molar implant 1 gave very severe biomechanical condition to the surrounding bone tissue. For both the models, the distance between the implants 1 and 2 was longest and therefore, the implant 1 needed to support wider region than the other implants, resulting in the highest load bearing situation. Furthermore, in the female model, the implant 1 was the shortest and therefore, the contact area with the surrounding bone was also smallest, resulting in such high concentration of SED. As a trial, an inclined longer implant was inserted into the location of the implant 1 in the female model and finite element analysis was then conducted to check the effect of the implant exchange on the SED distribution. It was found that the maximum SED was dramatically reduced and became about half of the previous value by introducing such implant exchange.

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Morphology and Differentiation of Human Periodontal Ligament Cells on Honeycomb Films

Nagayoshi Iwama, Takahito Kawano, Hiroshi Ishihata, Hidetoshi Shimauchi, and Masatsugu Shimomura

Abstract. Biocompatible honeycomb-patterned porous polymer films (HFs) were prepared by using self-organization process. This study was aimed to investigate the possibility of HFs as the cell scaffold for the periodontal regenerative therapy. Periodontal ligament (PDL) cells cultured on HFs changed the morphology and expressed osteopontin (OPN), suggesting the enhanced cell differentiation. HFs may be applicable to periodontal regenerative therapy as a functional scaffold.

Key words. Autotransplant, Honeycomb-patterned porous polymer film, Periodontal ligament cells, Periodontal regenerative therapy

1 Introduction

PDL contains stem cell-like populations which have pluripotent and self-renewal ability. PDL cells are considered to be an important cellular source in periodontal tissue engineering. However, it is also necessary to develop the most suitable scaffold which promotes cell proliferation and differentiation, to achieve complete regeneration. We have previously reported that HFs prepared by self-organization

N. Iwama (✉), H. Ishihata, and H. Shimauchi
Division of Periodontology and Endodontology, Tohoku University Graduate
School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: iwm@dent.tohoku.ac.jp

T. Kawano and M. Shimomura
Institute of Multidisciplinary Research for Advanced Materials, Tohoku University,
2-1-1 Katahira, Aoba-ku, Sendai, Japan

process have three-dimensional structures, which are with well-arranged and uniform-sized pores, and connected hollow network between pores [1]. This study is designed to investigate the topographical effects of HF on PDL cell morphology and differentiation.

2 Materials and Methods

HF were fabricated from polystyrene (PSt) and prepared the pores of 5 or 10 μm diameter. Flat PSt film and glass substrate were used as controls. Human PDL cells were maintained in α -MEM supplemented with 10% FBS and antibiotics. The cells were seeded on HF or controls (glass substrate and flat film) at density of 1.0×10^4 cells/cm² and cultured for 4 h up to 28 days. The specimens were observed using scanning electron microscope (SEM; Miniscope TM-1000, Hitachi) and immunofluorescence microscope (FSX100; Olympus).

3 Results and Discussion

SEM analysis revealed that PDL cells were firmly attached on the pillars of 5 μm pored HF at 4 h. In contrast, the cells dived into the pores on the 10 μm pored HF (Fig. 1). After 28 days cultivation, PDL cells were becoming multilayered on HF. By immunofluorescence microscopy, the cells on 10 μm pored HF were stained with osteopontin (OPN), which is known as a marker protein produced by osteoblasts. These results suggested that the HF could regulate the PDL cells differentiation by the topographical effects. Our results may indicate that HF were applicable to the periodontal regenerative therapy as a functional scaffold for the cell-sheet technique.

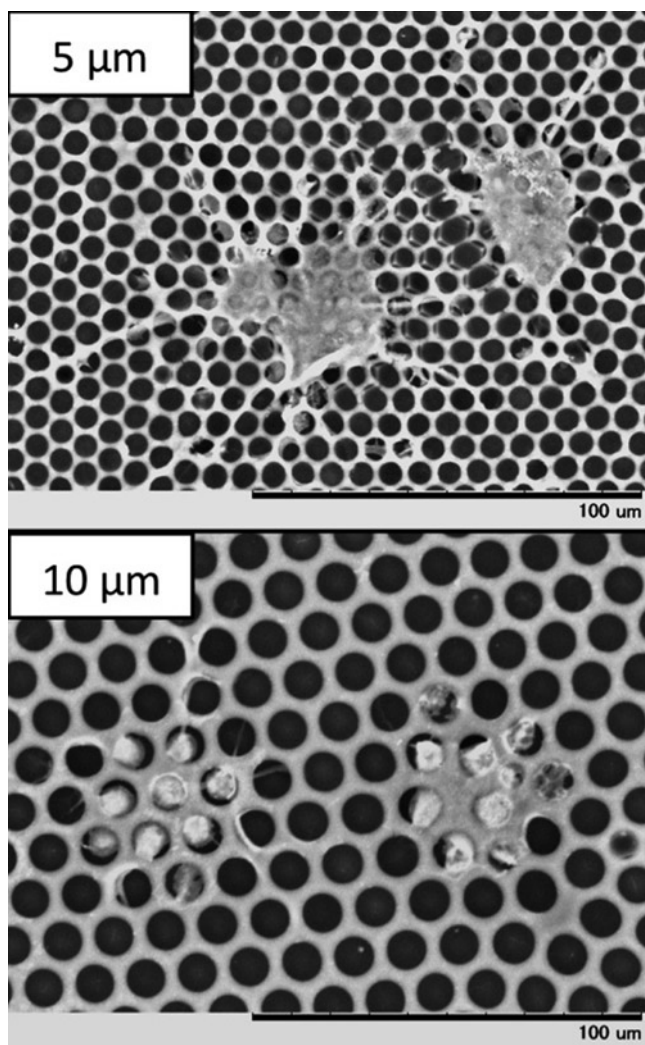


Fig. 1. SEM images of the PDL cells cultivated for 4 h on 5 and 10 μm pored HF. *Scale bar*, 100 μm

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Antagonism Between Bisphosphonates (BPs) and Structurally Related Substances in the Effects of BPs in Mice

Satoru Okada, Tomomi Kiyama, Takefumi Oizumi, Kouji Yamaguchi, Hiroshi Kawamura, Shunji Sugawara, and Yasuo Endo

Abstract. The anti-bone-resorptive effects (ABREs) of nitrogen-containing BPs (NBPs) are much more powerful than those of non-nitrogen-containing BPs (non-NBPs). However, NBPs have inflammatory and necrotic side effects (INSEs) including osteonecrosis of jaw bones. Here, we examined antagonism between BPs and structurally related substances in terms of ABREs and INSEs. Our findings suggest that a certain type(s) of transporters or related mechanisms may be involved in the intracellular uptake of NBPs, leading to INSEs.

Key words. Bisphosphonate, Clodronate, Etidronate, Osteonecrosis, Zoledronate

1 Bisphosphonates Are the Current First-Choice Drugs for Anti-Bone-Resorptive Effects

However, Bisphosphonate (BP)-related osteonecrosis of jawbones (BRONJ) has attracted much attention. BRONJ is caused largely by nitrogen-containing BPs (NBPs), whereas there are few reports concerning non-NBP-related BRONJ.

S. Okada (✉), T. Oizumi, and K. Yamaguchi
Department of Molecular Regulation, Graduate School of Dentistry, Tohoku University,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
and

Department of Oral and Maxillofacial Surgery, Graduate School of Dentistry,
Tohoku University, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: outsider4ki@yahoo.co.jp

T. Kiyama, S. Sugawara, and Y. Endo
Department of Molecular Regulation, Graduate School of Dentistry, Tohoku University,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

H. Kawamura
Department of Oral and Maxillofacial Surgery, Graduate School of Dentistry,
Tohoku University, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

We have reported that in mice: (a) zoledronate is the strongest BP in both anti-bone-resorptive effect (ABRE) and INSEs, and (b) clodronate and etidronate (both non-NBPs), which exhibit no apparent INSEs, antagonize the INSEs of NBPs, and etidronate (but not clodronate) antagonizes the ABREs of NBPs, too [1]. Here, we examined antagonism between BPs and structurally related substances in terms of ABREs and INSEs and obtained following results. (1) Oxidronate and medronate (both non-NBPs, used as carriers of ^{99m}Tc in bone-scintigraphy) exhibited ABREs comparable to those of clodronate and etidronate, respectively. (2) Like etidronate, medronate antagonized the ABRE, but not INSEs, of zoledronate. (3) Unexpectedly, oxidronate and medronate were inflammatory, and oxidronate by itself exhibited INSEs. (4) The INSEs of oxidronate were antagonized by clodronate and by etidronate, but not by medronate. (5) Pyrophosphoric acid partially antagonized the INSEs of oxidronate, but not those of zoledronate. (6) Glutamic acid and aspartic acid (with structures somewhat homologous to NBPs) partially antagonized or tended to antagonize the INSEs of zoledronate, but not those of oxidronate. These results represent the first demonstration that even if a given drug is a non-NBP, it may enter cells within soft tissues—depending on its precise configuration and/or that of a cell-surface component associated with its entry—resulting in toxic effects. Our data may provide important information for drug design in BP-therapy.

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Synthesis of Octacalcium Phosphate with Fluoride Ions and Protein Adsorption onto the Crystals

Yukari Shiwaku, Takahisa Anada, Yoshitomo Honda, Shinji Morimoto, Keiichi Sasaki, and Osamu Suzuki

Abstract. In this review, synthesis and protein adsorption of octacalcium phosphate (OCP) prepared with fluoride ions (F^-) were discussed. Two types of fluoride-containing calcium phosphates (F-CaPs) which partly preserve OCP crystal structures were produced. The F-CaPs exhibited different protein adsorption capacities regardless of F content in the solids. F-CaP prepared by hydrolysis (HF-CaP) adsorbed more amount of cytochrome c than F-CaP prepared by co-precipitation (CF-CaP) and original OCP. HF-CaP may have a capacity which can adsorb more osteogenic growth factors than original OCP does.

Key words. Co-precipitation, Fluoride, Hydrolysis, Octacalcium phosphate, Protein adsorption

Y. Shiwaku

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan and

Division of Craniofacial Function Engineering, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

T. Anada, Y. Honda, S. Morimoto, and O. Suzuki (✉)

Division of Craniofacial Function Engineering, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: suzuki-o@m.tains.tohoku.ac.jp

K. Sasaki

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

1 Introduction

Octacalcium phosphate (OCP) has been reported to have biodegradable property and facilitate bone regeneration [1, 2]. OCP is known to convert to fluoridated hydroxyapatite (FHA) immediately when fluoride ion (F^-) reacts with OCP [3]. It used to be difficult to prepare OCP with F^- , even though it may be effective to promote osteoblastic differentiation [4] and protein adsorption [5]. However, we succeeded in preparing fluoride-containing calcium phosphates (F-CaPs) which had OCP structure in part. The present article was focused on the description how F-CaPs affect protein adsorption on the crystals.

2 Synthesis Methods and Crystal Structures of F-CaPs

F-CaPs were synthesized by hydrolysis and co-precipitation methods [6]. F-CaP obtained by hydrolysis (HF-CaP) was carried out by incubating OCP in 150 mM Tris buffer (pH=7.1–9.9) containing 50 ppm F^- at 37°C. On the contrary, F-CaP obtained by co-precipitation (CF-CaP) was performed by mixing calcium solution with phosphate solution including 12 and 96 ppm F^- at 70°C.

X-ray diffraction (XRD) analysis indicated that HF-CaP prepared at pH=9.9 maintained the crystal structure of OCP whereas HF-CaP prepared at pH=7.1 completely converted to FHA. On the other hand, CF-CaP preserved the crystal phase of OCP in the preparation containing 96 ppm F^- , although FHA precipitation was accompanied.

3 Improvement in Protein Adsorption onto HF-CaPs

In order to analyze the protein adsorption on both F-CaPs, bovine serum albumin (BSA, pI=4.7) and cytochrome c (pI=10.2) [6] were used as model adsorbate proteins for growth factors. A 10 mg sample was mixed with 1 mL protein-dissolved buffer (pH=7.4) for 5 min. The amount of protein in the supernatant was measured by Dc protein assay (BIO-RAD).

The results showed that the amount of cytochrome c adsorbed were higher in HF-CaP than those in CF-CaP and OCP. On the contrary, BSA was more adsorbed on CF-CaP and OCP than that on HF-CaP. These results suggest that HF-CaP may work as a better adsorbent for basic proteins, such as osteogenic growth factors.

The differences in the protein adsorption onto F-CaPs may be stemmed from the surface physicochemical properties, such as electrostatic nature and the crystal morphology of F-CaPs. It is probable that the preparation method controls the growth of OCP and its conversion into FHA. Further study is underway to investigate the factors determining the adsorption property by both F-CaPs.

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Corrosion Resistance of Dental Metals to Hydroxyl Radical Generated by Photolysis of H_2O_2

Yasutomo Yamada, Keisuke Nakamura, Yukyo Takada, Takayuki Mokudai, Hiroyo Ikai, Ryoichi Inagaki, Taro Kanno, Keiichi Sasaki, Yoshimi Niwano, and Masahiro Kohno

Abstract. Corrosion resistance of dental metals to hydroxyl radical generated by photolysis of H_2O_2 is discussed in comparison with that to H_2O_2 alone in this review article.

Key words. Corrosion resistance, Dental metals, Hydrogen peroxide, Hydroxyl radical

1 Introduction

A novel disinfection technique for dental treatment has been developed in our laboratory [1]. A remarkable feature of this technique is the generation of hydroxyl radical by photolysis of H_2O_2 . It is known that hydroxyl radical is very strong oxidant for organic substances, so that it can oxidize cellular components of microorganisms leading to cell death [2]. However, little is known about its oxidative potential for inorganic materials, such as dental metals. In general, dental metals have high

Y. Yamada (✉), H. Ikai, R. Inagaki, T. Kanno, and K. Sasaki
Division of Fixed Prosthodontics, Department of Restorative Dentistry, Tohoku University
Graduate School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: yamada@s.tohoku.ac.jp

K. Nakamura, T. Mokudai, and Y. Niwano
Laboratory for Redox Regulation, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai, Japan

Y. Takada
Division of Dental Biomaterials, Department of Restorative Dentistry,
Tohoku University Graduate School of Dentistry, Sendai, Japan

M. Kohno
New Industry Creation Hatchery Center, Tohoku University,
6-6-10 Aoba, Aramaki, Aoba-ku, Sendai, Japan

corrosion resistance because they are used in oral cavity of which environment is corrosive. Nevertheless, there is concern about metallic corrosion of dental metals caused by strong oxidizing potential of hydroxyl radical when the disinfection technique is applied in oral cavity. The purpose of this review article is to discuss the corrosion resistance of various dental metals to hydroxyl radical generated by photolysis of H_2O_2 .

2 Static Immersion Test

Four different dental metals, Ag–Pd–Cu–Au alloy, pure titanium, Co–Cr alloy, and stainless steel (SUS316L), which are widely used in dental treatment, were used for the static immersion test. For instance, Ag–Pd–Cu–Au alloy is applied for metallic restorations such as inlays, artificial crowns, and bridges in Japan. Ti is applied for dental implants. Co–Cr alloy is applied for metal components of removable dentures. SUS316L is applied for dental instruments as well as orthodontic wire and bracket. The corrosion behavior of the dental metals in the hydroxyl radical generation system by photolysis of H_2O_2 was examined in comparison with that in H_2O_2 alone. The static immersion test was performed basically according to ISO 10271. The specimens were immersed in 1M H_2O_2 with or without LED-light irradiation at 400 nm for one week. Then, the released ions in the solution were analyzed by inductively coupled plasma optical emission spectrometry. When Ti was immersed in 1,000 mM H_2O_2 without LED-light irradiation, ion release was approximately $400 \mu\text{g}/\text{cm}^2\cdot\text{week}$. In contrast, ion release from other dental metals under any of the conditions was less than $100 \mu\text{g}/\text{cm}^2\cdot\text{week}$. In any case, the amount of released ions from each dental metal in the hydroxyl radical generation system never exceeded that in the H_2O_2 alone.

3 Time Course Changes in the Concentration of H_2O_2 and the Yield of Hydroxyl Radical

Electron spin resonance analysis revealed that 340 mM hydroxyl radical was generated in one week when the H_2O_2 was irradiated by the LED-light. The concentration of H_2O_2 decreased to around 200 mM under the same condition. On the other hand, the concentration of H_2O_2 was unchanged and only a small amount of hydroxyl radical was generated under the condition of H_2O_2 without the LED-light irradiation.

4 Conclusion

The dental metals used in the study were not corroded by the hydroxyl radical generated by H_2O_2 as severely as by H_2O_2 alone. It is suggested that the disinfection system would probably be used from a viewpoint of metal corrosion wherever 3% H_2O_2 can be used as a disinfectant.

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Accelerated Bone Formation Around Titanium Dental Implants with Amorphous Calcium Phosphate Coating in Rabbits

Sou Yokota, Jun Kurihara, Naruhiko Nishiwaki, Soichiro Tamate,
Kyosuke Ueda, Takayuki Narushima, and Hiroshi Kawamura

Abstract. The aim of this study was to investigate the bone response of titanium dental implant with amorphous calcium phosphate (ACP) coating film fabricated using RF magnetron sputtering in rabbit. Calcium phosphate coating using RF magnetron sputtering acquires high bonding strength and nano-uniformity of films. Moreover, it is reported that ACP was resorbed rapidly in vitro. The ACP-coated and non-coated implants were placed into rabbits. After 1, 2 and 4 weeks of implantation, the implant stability was evaluated by resonance frequency analysis (RFA) and bone formation was also evaluated histomorphometrically. The ACP-coated implants showed higher stability in RFA and improved bone-implant contact ratio in 4 weeks. Titanium dental implant coated with ACP fabricated using RF magnetron sputtering accelerated bone formation compared with that of non-coated implants.

Key words. Animal experiment, Calcium phosphate, Dental implant, RF magnetron sputtering

1 Introduction

Dental implants have been useful method and shown high survival rates. Such high success rates have been attributed to the intimacy of bone and implant interface called “osseointegration”. Moreover, it has been suggested that titanium (Ti) dental implants with calcium phosphate (CaP) coating acquire faster bone growth

S. Yokota (✉), J. Kurihara, and H. Kawamura
Division of Maxillofacial Surgery, Department of Oral Medicine and Surgery,
Graduate School of Dentistry, Tohoku University, Sendai 980-8575, Japan
e-mail: sos@dent.tohoku.ac.jp

N. Nishiwaki, S. Tamate, K. Ueda, and T. Narushima
Department of Materials Processing, Tohoku University,
6-6-2 Aoba, Aramaki, Aoba-ku, Sendai 980-8579, Japan

compared with non-coated. However, the phase and microstructure of CaP coatings affect bone-forming ability of implants. We reported that dense and uniform calcium phosphate coating film with high bonding strength to Ti could be obtained using RF magnetron sputtering [1] and found that amorphous calcium phosphate (ACP) coating film was bioresorbable.

2 Materials and Methods

ACP film was coated onto Ti dental implants (Nobel Biocare, Gothenburg, Sweden) using RF magnetron sputtering. The ACP-coated and non-coated implants were placed into femur and tibia of New Zealand white rabbits for up to 4 weeks. The 72 implants were given to 18 rabbits. After 1, 2 and 4 weeks, the effect of ACP coating was evaluated by RFA (resonance frequency analysis) using Ostell (Integration Diagnostics Ltd, Gothenburg, Sweden) and bone formation was also evaluated histomorphometrically using undecalcified sections of removed bone-implant block.

3 Results

3.1 Resonance Frequency Analysis

After 1, 2 and 4 weeks of implantation, the result of implant stability quotient (ISQ) values were measured by RFA in femur. The ISQ values increased with implantation time, suggesting the osseointegration would be acquired. There was no significant difference in 1 and 2 weeks between ACP-coated and non-coated implant, but there was significant difference in 4 weeks ($p < 0.05$).

3.2 Bone-Implant Contact Ratio

The result of bone formation was expressed as bone-implant contact ratio (BIC). All specimens demonstrated bone formation directly on the implants surface without any particular difference between implants and adjacent tissue. In 2 and 4 weeks after implantation, ACP-coated implants showed significantly increased BIC compared to non-coated (Table 1).

Table 1 There was no significant difference between the ACP-coated and non-coated in 1 week. But at the 2 and 4 week, the ACP-coated groups were significantly higher than the non-coated group ($p<0.01$)

	1 week		2 week		4 week	
	Mean	SD	Mean	SD	Mean	SD
Non-coated	18.26	5.27	49.31	15.05	64.76	14.45
ACP-coated	20.23	5.9	63.41	11.6	78.49	8.83
	p -value 0.29		p -value<0.01		p -value<0.01	

4 Conclusion

ACP coating film fabricated by RF magnetron sputtering on titanium dental implants accelerated bone formation compared with that of non-coated implants.

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Hydroxyapatite Film for Dental Treatment by Powder Jet Deposition: A Review

Ryo Akatsuka, Hiroshi Ishihata, Miyoko Noji, Ken Matsumura,
Takahiro Anada, Tunemoto Kuriyagawa, Osamu Suzuki,
and Keiichi Sasaki

Abstract. This review summarized the powder jet deposition (PJD) method used to create a thick hydroxyapatite (HAp) film on a human tooth surface. The PJD method is a technology for creating ceramic films on ceramic substrates in which fine particles of ceramic were accelerated and the only deposition process that could be used in the room temperature and room pressure. The HAp film created in previous studies showed that excellent microstructure and mechanical properties. The possibility of making a new interface between the tooth and biomaterials using the PJD method was indicated.

Key words. Clinical application, Hydroxyapatite film, Powder jet deposition, Tooth–biomaterial interface

R. Akatsuka (✉), M. Noji, K. Matsumura, and K. Sasaki
Division of Advanced Prosthetic Dentistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: akatsuka@dent.tohoku.ac.jp

H. Ishihata
Division of Periodontology and Endodontology, Tohoku University Graduate
School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

T. Anada and O. Suzuki
Division of Craniofacial Function Engineering, Tohoku University Graduate
School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

T. Kuriyagawa
Department of Nanomechanics, Tohoku University Graduate School of Engineering,
6-6-1 Aramakiyaza-aoba, Aoba-ku, Sendai 980-8579, Japan

1 Introduction

Biomaterials used for dental restorative treatments have different chemical compositions and mechanical properties from tooth substances [1]. Even if dental adhesion techniques are properly applied, these differences cause clinical problems like drop-off of the restorative materials from tooth, secondary caries. These problems can be solved by creating a film between the tooth surface and biomaterials as an interface. The HAp film can be used to generate a novel interface between the tooth and biomaterials in dental restorative treatment because the HAp is chemically and mechanically stable bioceramic.

2 Development of PJD Method

Numerous experimental deposition processes have been investigated, including thermal spraying, sputter coating, pulsed laser ablation, dynamic mixing, dip coating, sol-gel, electrophoretic deposition, biomimetic coating, hot isostatic pressing and aerosol deposition [2]. In most of these processes, the substrate needs to be heated and processed in a chamber under a vacuum. In contrast, PJD is carried out at room temperature and atmospheric pressure, which makes it possible to use it with HAp particles to create a HAp film on human tooth.

The PJD technique originated in abrasive jet machining (AJM), which is one of the precise processing methods for hard-brittle materials through an average particle size of about 10–20 μm , and it is also a well known non-contact mechanical removal process. When smaller particles of ceramic or metal, about 1–3 μm in diameter, were blasted onto ceramic substrate using an AJM device, the particles were deposited on the substrate. This is called the PJD technique [3].

3 Evaluation of the HAp Film

Previous studies showed that HAp particles were deposited firmly as a HAp film on human enamel substrate by the PJD technique [3, 4]. The average thickness was about 30 μm and sixth maximum thickness was about 40 μm . HAp particles were densely packed, and the shape of each HAp particle in the HAp film had been highly deformed from the original shape. There was no obvious gap between the HAp film and the enamel substrate. The micro-Vickers hardness of the HAp film was as same as that of the enamel. The bonding strength of HAp film that showed the Type 2 fracture pattern was the same as the bonding strength between the composite resin and the enamel substrate [4]. Their permeability of the dentin was indirectly recorded by a split chamber device and by using a chemiluminescence technique [5] and the permeability after application of the HAp films was significantly lower than that of the no-treated controls.

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Growth Behavior of Mesenchymal Stem Cell in 3D Porous Collagen Scaffolds

Takaaki Arahira, Mitsugu Todo, and Gouping Chen

Abstract. In this study, porous structures of pure collagen and collagen/ β -TCP composite material were developed as scaffolds for bone tissue engineering. Rat bone marrow mesenchymal stem cells (rMSC) were then cultured in the scaffolds with α -MEM based medium and a differentiation supplement. It was found that the compressive moduli of the scaffolds with rMSCs tended to increase with progress of proliferation and differentiation of the cells. Collagen/ β -TCP composites were considered to be better scaffold material than pure collagen.

Key words. Collagen scaffold, Compressive modulus, Mesenchymal stem cell, Regenerative medicine

1 Introduction

Recently, in the field of bone tissue engineering, regenerated bone graft has been one of the primary concerns instead of autografts and allografts [1]. A regenerated graft may be developed by culturing and differentiating mesenchymal stem cells in a porous material called scaffold [1]. In this case, the biochemical and biomechanical

T. Arahira
Interdisciplinary Graduate School of Engineering and Science,
Kyushu University, 6-1 Kasuga-koen, Kasuga 816-8580, Japan

M. Todo (✉)
Research Institute for Applied Mechanics, Kyushu University,
6-1 Kasuga-koen, Kasuga 816-8580, Japan
e-mail: todo@riam.kyushu-u.ac.jp

G. Chen
Biomaterials Center, National Institute for Materials Science,
Tsukuba, Ibaraki 305-0044, Japan

culture conditions and the structures and compositions of the scaffold are important factors controlling the quality of the regenerated bone graft. Natural polymers such as collagen have widely been used for scaffolds in tissue engineering [2]. Collagen guarantees excellent biological conditions, for example, it stimulates proliferation and differentiation of cells as extracellular matrix [3].

In this study, rat bone marrow mesenchymal stem cells (rMSC) were cultured in pure collagen and collagen/ β -TCP composite scaffolds up to 28 days. Compressive modulus and cell number were then evaluated and the growth behavior of rMSCs in these scaffolds were assessed on the basis of these properties.

2 Materials and Methods

Porous collagen and collagen/ β -TCP composite scaffolds were fabricated by the freeze-drying method. Collagen solution and a mixture of collagen and β -TCP powders (the weight ratio=90:10) in the solution were used to fabricate the scaffolds. The rMSC cells were then cultured in the scaffolds with an α -MEM based medium with a differentiation supplement up to 28 days. Compression tests of the scaffolds with proliferated cells were conducted periodically to evaluate compressive mechanical properties such as the stress-strain relation and the elastic modulus. Variations of cell number and ALP activity were also evaluated periodically. The porous microstructures and the proliferation behavior were characterized by a field emission electron microscope (FE-SEM).

3 Results and Discussion

In the collagen/ β -TCP scaffold, the modulus tended to increase with increase of culture period, while the modulus of the pure collagen scaffold did not show such change. Actually, the modulus of the pure collagen scaffold tended to decrease up to 14 days and then recovered to the almost same value with the initial modulus at 28 days. Those changes of the macroscopic moduli of the scaffolds with cells are thought to be strongly related to the degradation behavior of collagen and the proliferation and differentiation of rMSC. It is noted that the differentiation of rMSCs to osteoblasts resulted in the formation of collagen and calcification and therefore, tended to increase the macroscopic modulus.

In conclusion, the compressive elastic moduli of the collagen/ β -TCP scaffold tended to increase with increase of culture period and this is mainly due to the cell proliferation, differentiation and subsequent ECM formation and calcification. The modulus of the pure collagen scaffold tended to decrease up to 14 days due to the degradation of the scaffold and then increase up to 28 days because of the proliferation.

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The Surface Wettability of Trial Acrylic Denture Base Resins

Maimaitishawuti Dilinuer, Guang Hong, WeiQi Wang,
Taizo Hamada, and Keiichi Sasaki

Abstract. This study aimed to influence of sericin powder on surface wettability of trial acrylic denture base resins. The results suggest that the addition of sericin powder to the powder form can improve the surface wettability of acrylic denture base resin.

Key words. Acrylic denture base resin, Sericin powder, Surface wettability

1 Background

Surface wettability of denture base participates in denture retention, stability during oral function significantly. Improvement of wettability of denture base materials, therefore, can improve the denture function, which leads to upgrade of the quality of life (QOL) of aged denture wearers. The purpose of this study was to evaluate the surface wettability of experimental production of acrylic denture base resins containing sericin powder.

M. Dilinuer, W. Wang, and K. Sasaki
Division of Advanced Prosthetic Dentistry, Graduate School of Dentistry,
Tohoku University, 4-1 Seiryomachi, Aoba-ku, Sendai, Japan

G. Hong (✉)
Liaison Center for Innovative Dentistry, Graduate School of Dentistry,
Tohoku University, Sendai, Japan
e-mail: hong@m.tohoku.ac.jp

T. Hamada
Department of Oral Health Care Promotion, Graduate School of Dentistry,
Tohoku University, Sendai, Japan

2 Material and Methods

In this study, two methyl methacrylate polymer (D-100M and D-250ML) with different molecular weight and particle size, one methyl methacrylate/ethyl methacrylate copolymer (D-300), and one ethyl methacrylate polymer (D-250E) were used in powder form with contained 3wt% sericin powder (Kashiro Sangyo Co.). The methyl methacrylate (MMA), butyl methacrylate (i-BMA), methacrylate 2-ethylhexyl (EHMA) and methacrylic 2-hydroxyethyl (HEMA) were used in liquid form. The ethylene glycol dimethacrylate (EGDMA) and tri-ethylene glycol dimethacrylate (TEGDMA) were used as a crosslinking agent. The surface wettability of materials were evaluated using a portable contact angle meter (PCA-1, Kyowa Interface Science Co.) after immersion in water, 0, 1, 2, 3, 7, 14, 30 and 90 days at 37°C. Statistical analysis was performed using 1-way analysis of variance (ANOVA), Student–Newman–Keuls multiple comparison tests (S–N–K test) ($\alpha=05$).

3 Results and Discussion

In initial contact angle of each material, no significant difference was found among the each formulations ($p>0.05$, ANOVA) (Fig. 1). The contact angle of all samples significantly decreased with increases in immersion time ($p<0.05$, one-way ANOVA) (Fig. 2). From 30 days after immersion, the contact angle of most samples significantly increased with increases in immersion time (Fig. 2). After immersion, the material which used 250-E for powder showed lowest values of contact angle than the other formulations (Fig. 2).

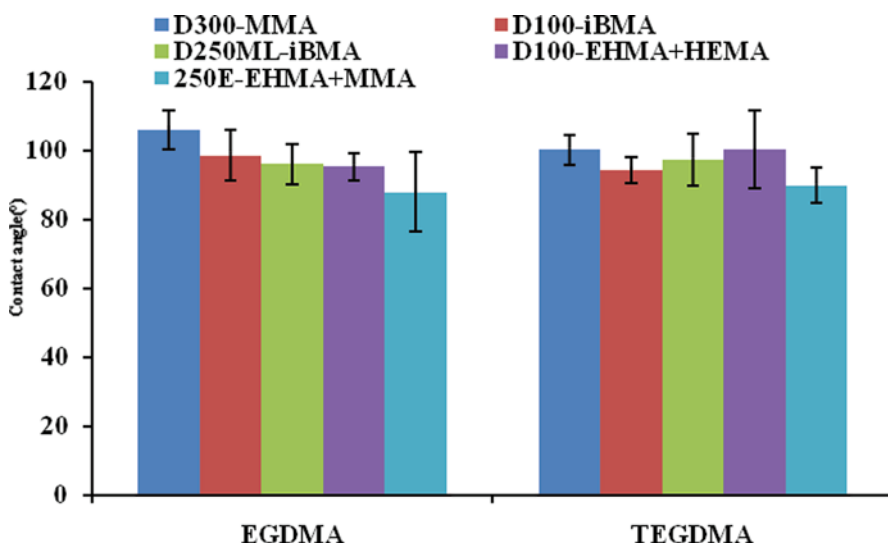


Fig. 1. Initial contact angle of materials

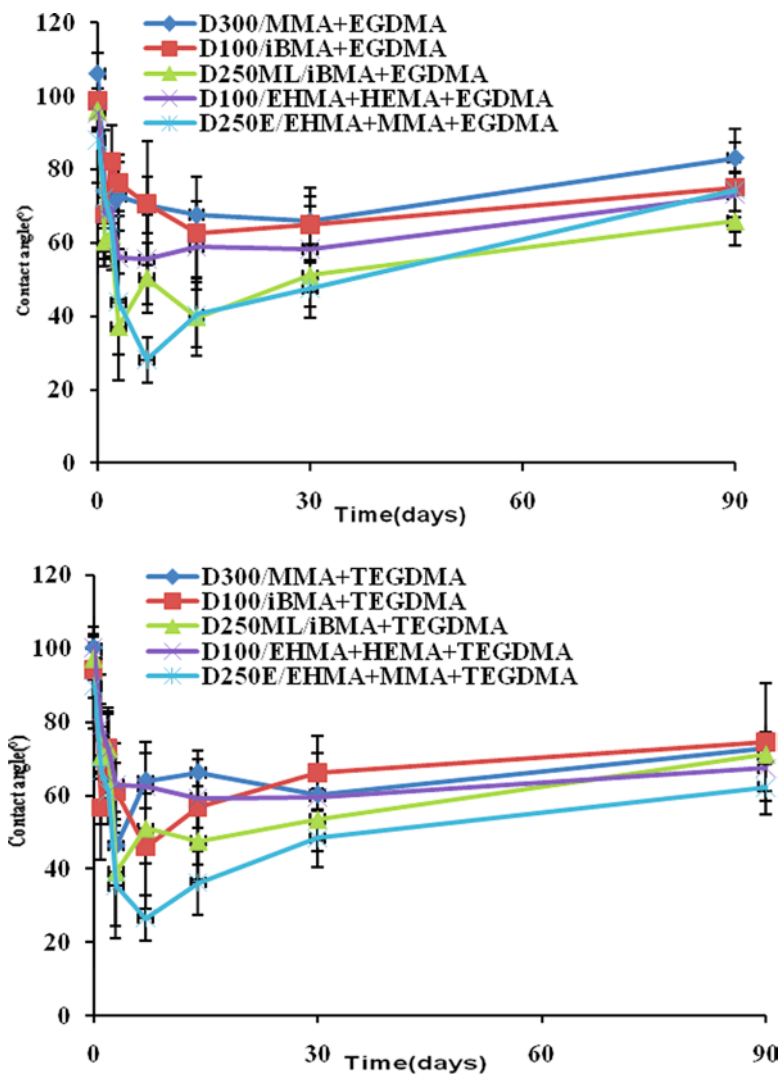


Fig. 2. The contact angle of materials after immersion

4 Conclusions

The suitable combination of the components of acrylic denture base resin, and the addition of sericin powder to the powder form can improve the surface wettability of these materials.

Microstructural and Mechanical Characterization of the Porous Anodic TiO₂ Layer on Titanium

Zhao-Xiang Chen, Wen-Xue Wang, Yoshihiro Takao,
Terutake Matsubara, and Li-Mei Ren

Abstract. Porous TiO₂ layer was fabricated on the titanium surface using an anodic spark oxidation technique for biomedical application. The microstructural and mechanical characterization of TiO₂ layer was performed. Results show that the fabricated TiO₂ layer exhibited a porous surface and poorly-crystallized structure, and an inner layer containing numerous cavities was observed near the oxide-substrate interface. The mechanical characteristics of TiO₂ layer were greatly influenced by its microstructural features. The performed shear test showed that the shear fracture of oxide layer took place primarily within the cavity-containing layer. The performed tensile fatigue loading resulted in multiple cracking to occur in the oxide layer with distinctive inclined cracking driven by slip band in the titanium substrate, and localized spallation of oxide layer was also observed. Despite extensive fatigue damage in the oxide layer, the fatigue life of titanium was not significantly influenced by the present anodization treatment.

Key words. Anodic spark oxidation, Fracture, Microstructure, Titanium

1 Introduction

Anodic spark oxidation treatment has been currently applied to titanium implant for biomedical application [1]. Although the corrosion behaviors and biocompatibility of spark anodized titanium have been widely studied [1–3], little is known about the mechanical properties of anodic TiO₂ layer on the titanium surface. The purpose of this study was to investigate the microstructural and mechanical characteristics of

Z.-X. Chen (✉), W.-X. Wang, Y. Takao, T. Matsubara, and L.-M. Ren
Research Institute for Applied Mechanics, Kyushu University,
Kasuga-koen 6-1, Kasuga, Fukuoka 816-8580, Japan
e-mail: zxchen@live.com

TiO₂ layer fabricated by anodic spark oxidation technique, especially its shear and fatigue fracture characteristics, so as to provide valuable references for the design, performance and life prediction of such TiO₂ layer in medical implants.

2 Material and Methods

The ground titanium specimens and platinum plates were connected to the anode and the cathode respectively, and anodic spark oxidation was carried out in 1 M phosphoric acid solution for 2 min at a voltage of 200 V using a DC power supply. The microstructural features of TiO₂ layer were investigated by SEM, TEM and XRD. The shear fracture characteristics of TiO₂ layer were studied via a lap-shear test. The fatigue testing was performed at a frequency of 10 Hz with a stress ratio of $R=0.1$ under a tension–tension stress mode. The maximum applied cyclic stress ranged between 120 and 160 MPa.

3 Results and Discussion

The present anodic spark oxidation of titanium resulted in a porous amorphous TiO₂ layer with many microprojections and craters on its surface. The craters were formed by dielectric breakdown, which caused the recession of the oxide/substrate interface. Such recession results in a relatively rough oxide/substrate interface and thus has an enhanced effect on the interfacial shear strength due to mechanical interlocking. TEM cross-sectional observation of TiO₂ layer disclosed a cavity-containing layer along the oxide/substrate interface, which potentially weakens the interfacial adhesion strength. During the shear test, the shear fracture of TiO₂ layer took place primarily within the cavity-containing layer. During tensile fatigue loading, significant surface damage was induced in the TiO₂ layer. At relatively low stress level (maximum cyclic stress = 120 MPa), small and narrow cracks perpendicular to the tensile direction were formed in the oxide layer. When the maximum cyclic stress was increased to 140 MPa, cracking and spallation of oxide layer at inclined angle of about 45° was observed, which was initiated by slip bands developed in the titanium substrate. The fatigue life of titanium was not significantly affected by the present anodic spark oxidation treatment and could be estimated to be 140 MPa for both untreated and treated titanium.

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Improvement of the Dentin Bond System by Reducing the Oxygen Inhibition Effect on Curing of Resin-Based Bond Materials

Tatsuo Endo, Tamami Hoshino, Hiromi Sasazaki,
Etsuko Miura, and Masashi Komatsu

Abstract. The purpose of this study was to determine effects of light curing of self-etching adhesives under ambient air or argon on inhibition depths and dentin bond strengths. Curing under ambient argon significantly reduced oxygen inhibition at the free specimen surface and increased tensile bond strengths (TBS) to dentin. Especially, MGF showed the largest inhibition of polymerization in ambient air, but showed the highest improvement of TBS under ambient argon.

Key words. Argon, Dentin bond strength, Oxygen inhibition

1 Introduction

It is well known that polymerization will be inhibited by oxygen in many resin materials [1, 2]. In order to solve the mechanism of adhesion to tooth substance in detail, the adhesion test was performed in the state where the polymerization inhibition by oxygen was eliminated as much as possible.

2 Materials and Methods

2.1 Materials Used

Adhesives investigated: IMB-3(IMB, Tokuyama, Japan), AQ Bond Plus (AQP, Sun Medical, Japan), and Megabond FA (MGF, Kuraray, Japan).

T. Endo (✉), T. Hoshino, H. Sasazaki, E. Miura, and M. Komatsu
Division of Operative Dentistry, Department of Restorative Dentistry, Tohoku University
Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: endouta@m.tohoku.ac.jp

2.2 Inhibited Layer Thickness Measuring

Small droplets of the adhesives were placed on a microscopic glass slide between two spacer plates, 0.17 mm thick, under a cover-glass plate supported by the spacers to produce uniformly thick disc-shaped specimens. Soon after light activation by using Glip LightII for 10 s, the depths of oxygen inhibition were determined on six discs of each of the adhesives, when cured between the glass plates either under ambient atmosphere or a gentle stream of argon.

Inhibited layer thickness (ILT) was microscopically measured by using ECLIPSE 80i with Imaging Software (NIS-Elements, Nikon, Japan), on 6 disk-shaped specimens each. Statistical data analyses: Two-factor factorial ANOVA at $P < 0.01$ and Welch's t-test at $P < 0.05$.

2.3 Tensile Bond Strengths Test

The dentin surfaces were exposed and flattened using the carborundum wet-grinding paper #320. The flattened teeth surfaces were treated according to the manufacturer's instructions and the stainless steel rings (inner $\Phi = 4$ mm, height = 2.5 mm) were fixed on the surfaces. Immediately after the application of the bonding materials, the rings were filled with the light curing composite (Clearfil AP-X: Kuraray, Japan) and irradiated using Glip LightII for 60 s. Thirty minutes after the application of the bonding agents, the specimens were immersed in distilled water and stored at 37°C. Tensile bond strength (TBS) on dentin ($n = 6$) was measured at cross head speed of 0.5 mm/min 24 h after immersing in distilled water. Statistical data analyses: Two-factor factorial ANOVA at $P < 0.01$ and Welch's t-test at $P < 0.05$.

3 Results and Discussion

3.1 Inhibited Layer Thickness

Adhesive curing under ambient air revealed significant ILT (μm) differences: AQP (2.24) < IMB (3.46) < MGF (5.09). Upon argon curing limited inhibition occurred with AQP (0.31), IMB (0.42) and MGF (0.55).

3.2 Tensile Bond Strengths

Two-factor factorial ANOVA results revealed that TBSs in MPa (activation under air/argon) were significantly different between ambient air or argon and materials.

IMB: 10.3/12.5. AQP: 12.3/12.8. MGF: 16.1/21.1. TBSs on dentin, only MGF showed higher TBS after argon curing ($P=0.015$) than in ambient air.

When analyzed by two-factor factorial ANOVA, TBSs between under air and argon groups were significantly different. In the short-term results in a laboratory, even if there are small differences, the long-term clinical results will be greatly affected. By carrying out the polymerization of the resin in a argon atmosphere, the polymerization of the resin in an adhesion interface becomes more advanced and increases durability of bond effect.

4 Conclusion

Curing under ambient argon significantly reduced oxygen inhibition at the free specimen surface and increased the bond strengths to dentin. MGF showed the largest inhibition of polymerization in ambient air, but showed the highest improvement of TBSs under ambient argon.

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Curing Depth of New Sister Resin Composites of High and Low Consistency

Tamami Hoshino, Masafumi Kanehira, Werner J. Finger,
and Masashi Komatsu

Abstract. Aim of this study was to investigate and compare the depth of cure of two recently introduced flowable composites, MI FIL (MFI) and MI Flow (MFL), with two conventional and three nanofiller containing composites. For Knoop hardness (KHN) profiling specimens were produced in split molds. Hardness indentations were made at 250 μm distance along the center line and two parallel lines, 0.5 mm apart. KHN of all resins decreased with depth. MFI showed higher KHN than MFL at all depths. All hardness profiles followed second order polynomials with co-efficients $r^2 > 0.955$ ($p < 0.001$). Twenty seconds light activation proved insufficient to reach 2 mm depth of cure with the flowable resin composites MFI and MFL. From the seven resin restoratives tested only two were cured to a depth of ≥ 2 mm, when 80% of the top hardness is considered a relevant threshold for sufficient cure.

Key words. Curing depth, KHN, Resin composite

1 Introduction

The depth of polymerization of resin composites is commonly determined using the ISO 4049 scraping method or consecutive hardness measurements along the axis of specimens. Apart from output characteristics of the curing unit, the obtainable depth of cure depends on monomer composition, filler type, size and volume loading, initiating system, resin shade and opacity, and distance of the light tip from the target surface. Recently, two sister resin composites from the same manufacturer (GC)

T. Hoshino (✉), M. Kanehira, W.J. Finger, and M. Komatsu
Division of Operative Dentistry, Department of Restorative Dentistry,
Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi,
Aoba-ku, Sendai 980-8575, Japan
e-mail: tamami-th@umin.ne.jp

have been introduced to the Japanese market. The main difference between these materials is apparently the average filler particle size.

Aim of this in vitro trial was to determine and compare by hardness profiling the depths of cure of the two related and five alternative resin composites.

2 Materials and Methods

The related GC composites tested were MI Fil (MFI) and MI Flow (MFL). The nano-hybrid products were Filtek Supreme XT (FIL), Kalore (KAL), and Venus Diamond (VED). The microfilled Durafill VS (DUR) and the micro-hybrid Filtek Z250 (Z250) served as references. For hardness profiling specimens were produced in split molds ($3 \times 2 \times 6$ mm), filled with excess and pressed flush prior to 20 s LED light activation ($1,200 \text{ mW/cm}^2$). Knoop indentations were made at $250 \text{ }\mu\text{m}$ distance along the center line and two parallel lines 0.5 mm apart, starting $250 \text{ }\mu\text{m}$ underneath the edge of the top surface. Depth of cure was defined as the depth underneath the irradiated surface in mm, where the Knoop hardness (KHN) according to polynomial regression was 80% of the calculated maximum top surface hardness.

3 Results

The Knoop hardness (KHN) of all resins decreases with specimen depth, following second order polynomials with coefficients $r^2 > 0.955$ ($p < 0.001$). MFI shows higher hardness than MFL at all depths. Eighty percent of the materials' calculated top hardness and corresponding curing depths (KHN/mm) were: MFI: 30.7/1.58; MFL: 22.3/1.20; Z250: 49.8/1.52; DUR: 14.8/2.25; FIL: 58.1/1.96; KAL: 33.0/2.19; VED: 41.4/1.45.

4 Discussion and Conclusion

In this trial 80% of the top surface KHN was selected as a threshold value for reasonable and sufficient depth of cure of 2 mm thick composite layers. Apart from KAL and DUR the curing depth resulting from 20 s activation was less than 2 mm at 80% KHN.

Increasing the filler volume generally increases composite hardness. However, in addition also the filler grain size and the photoinitiator type may have so far underestimated effects. It is not fully elucidated yet whether scattering of light at nano-filler particles will affect the depth of cure. MFI and MFL are very similar products with same filler loading in weight percent. Reducing the average filler grain size from $0.7 \text{ }\mu\text{m}$ (MFL) to $0.2 \text{ }\mu\text{m}$ (MFI) has a noticeable effect on hardness of the

cured materials. The low depth of cure obtained with MFL at 80% KNH is probably not critical, as this product is indicated for lining of cavities or restoration of minimally invasive cavities only.

Practitioners should consider higher radiant exposure than recommended by manufacturers for more effective curing of resin composites at ≥ 2 mm layer thickness.

Finite Element Analysis to Determine the Stress Distribution in Implant Components

Ryoichi Inagaki and Masanobu Yoda

Abstract. A dental CAD/CAM system for implant prostheses in which abutments and superstructures are machined all together has been developed in our laboratory. For the fabrication process, analysis of a stress caused by occlusal force in the implant system is essential to establish an appropriate design of the prostheses in terms of biomechanics. Finite element analysis (FEA) has been recognized as a useful method which predicts the effect of stress on implant. The purpose of the present study was to analyze the stress distribution in the implant components using FEA and to determine a biomechanically ideal shape of the implant prostheses machined by the CAD/CAM system. The maximum stress values for the internal and external joint type are 1,400 and 2,000 MPa, respectively. It was demonstrated that the internal joint type could effectively resist to the stress, and reduce the stress value by 30% compared with the external joint type.

Key words. Dental CAD/CAM, External joint type, FEA, Implant, Internal joint type

1 Introduction

A dental CAD/CAM system for implant prostheses in which abutments and superstructures are machined together has been developed in our laboratory. For the fabrication process, analysis of the stress caused by an occlusal force in the implant system is essential to establish the appropriate biomechanical design of the prostheses. Finite element analysis (FEA) using a three-dimensional geometric model of

R. Inagaki (✉) and M. Yoda

Division of Fixed Prosthodontics, Department of Restorative Dentistry, Tohoku University
Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: inagaki@m.tohoku.ac.jp

the implant components has been recognized as a useful method to predict the effect of stress on the implant. The purpose of the present study was to conduct an FEA of the stress distribution in the implant components and to determine the biomechanically ideal shape of the implant prostheses machined by the CAD/CAM system.

2 Materials and Methods

FEA was performed assuming an implant system (Re, GC Co., Tokyo, Japan) with either an internal or an external abutment and fixture connection. The abutment had a 3-degree taper on one-third of the cervical part and a 2 mm vertical interval of the scallop. The superstructure for central incisors was designed with a 0.3 mm-thick metal frame. The thickness of the porcelain was 1.5 mm in the bucco-lingual direction and 2.0 mm at the proximal and incisal edges. The NX6 computer software (Siemens Product Lifecycle Management Software, Inc., Plano, TX) was used to design the three-dimensional geometric implant model. The NX6 Advance Simulation was used for the mesh data creation. Loading condition was applied to the incisal edge at an angle of 45° with 200 N assuming the maximum force.

3 Results

The stress concentration was not observed at the outer surface of abutment or the frame of superstructure. The highest stress concentration was observed at the top of screw where the abutment, the implant, and the screw were contacted. The maximum stress values for the internal and external type were 1,400 MPa and 2,000 MPa, respectively. The stress in the internal type was approximately 30% less than that in the external ones. In the internal type, the internal part of abutment which is inserted into the implant fixture dispersed the stress. The stress analysis like the present study needs establishment of precise geometrical model of the implant complex and analysis using three-dimensional model has been standardized as a results of some reports [1, 2]. These studies aimed at evaluation of screw loosening and reported that the internal type could resist the screw loosening though the stress concentrated at the cervical of screw. The results of the present study were not possible to determine the functional shape of the abutment and the frame of superstructure because the stress concentration was observed at neither the outer surface of abutment nor the frame. In the future, more precise analysis with different loading direction should be performed.

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Evaluation of the Accuracy of CAD/CAM Crowns Fabricated Using CT Images

Hiroaki Katoh, Shin Kasahara, Yoshinori Ebihara, and Kazuo Kikuchi

Abstract. In this study, from the standpoint of prevention of infection for dental technicians, we show the fabrication methods of crowns without impressions and dental model. We showed fabrication method of CAD/CAM crown using CAD data, and investigated the accuracy of the crowns. The discrepancies between the dies and crowns ranged from 50–95 μm at the margin and 15–40 μm at the shoulder in crowns fabricated from CT images of an abutment tooth. This present study showed a clinical utility of CAD/CAM crown that fabricated using CT images.

Key words. CAD/CAM, Crown, CT images, Infection

1 Introduction

In a previous study, with the goal to prevent infection for dental technicians, we propose a fabrication method of crowns without impressions or dental models. In this study, we investigated the accuracy of the CAD/CAM crowns using CAD data.

H. Katoh (✉)

Dentistry Sector, Tohoku University Hospital, 1-1 Seiryō-machi, Aoba-ku,
Sendai 980-8574, Japan
e-mail: h-katoh1010@dent.tohoku.ac.jp

S. Kasahara

Medical IT Center, Tohoku University Hospital, Sendai, Japan

Y. Ebihara

Advanced Technologies Development Center GC, Tokyo, Japan

K. Kikuchi

Comscantecno Corporation, Yokohama, Japan

2 Materials and Methods

A stone die with a height of 6 mm, diameter of 10 mm, shoulder width of 1 mm, and taper of 7° was used as an abutment model. CT images were taken using a CT scanning machine (Scan Xmate-D150S270, Comscantecno Corp, Yokohama). Measured CT data were converted to STL files and transferred to a CAD/CAM machine. Crowns were fabricated from composite blocks using a dental CAD/CAM system. Under the instructions of the manufacturer, $60\text{ }\mu\text{m}$ cement space was set at 1 mm upwards from the inner margin to the occlusal plane of the die.

The space between crowns and abutment dies was measured as the thickness of silicone fitness test material (GC Fit CheckerII). After mixing this test material, 147 N was applied as a load while material set. After setting, the silicone film was removed from the crown, and the thickness of the film was measured at the vertical cross section using a reflecting metallurgical microscope. Three samples were used to measure the fitness.

The measured results were compared with the fitness of conventional CAD/CAM crowns. Tests of the mean difference (independent t-test) at each measurement site were performed.

3 Results

The measured results are shown in Fig. 1 with statistical analysis. The white bar represents the conventional CAD/CAM crown, and the meshed bar represents the CT-CAD/CAM crown.

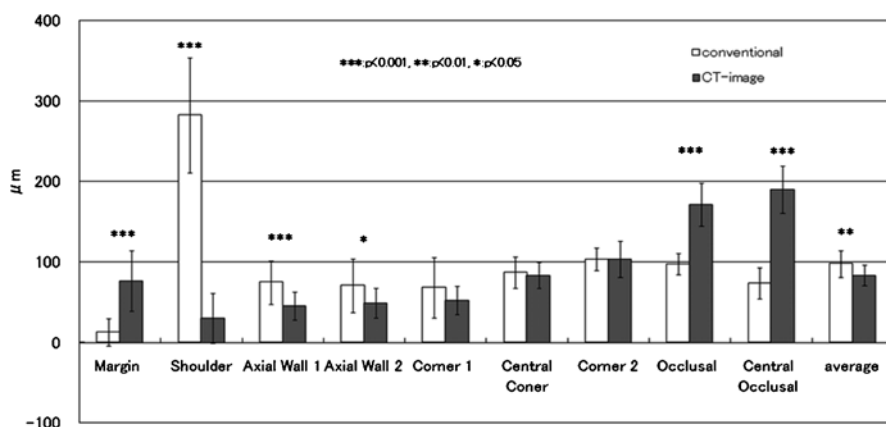


Fig. 1. Gap distances between crown and die after seating

On conventional cast crown, discrepancies of the die and cast crown are varied by location; at the shoulder the discrepancy is small, at the occlusal portion the discrepancy is large. However, discrepancies of the die and crown of the CT-CAD/CAM crown are uniform than that conventional CAD/CAM crown.

4 Conclusion

The discrepancies between the dies and crowns ranged from 50–95 μm at the margin and 15–40 μm at the shoulder in crowns fabricated from CT images of an abutment tooth. The gap at the axial wall was 50 μm in all specimens. The space at the occlusal region increased at the center from the axial-occlusal angle. This tendency is contrary to that observed in conventional CAD/CAM crowns [1, 2]. The results of this study indicated that the CT-CAD/CAM crown has higher accuracy than a conventional CAD/CAM crown.

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Effect of Zirconia Frame Thickness on Fracture Toughness of Veneer Porcelain

Momoko Kudo, Shoko Miura, Masafumi Kikuchi, Ryoichi Inagaki, Joonho Cho, Keiichi Sasaki, and Masanobu Yoda

Abstract. This review article focused on the influence of the zirconia thickness on the strength of the veneer porcelain, which was evaluated with a fracture toughness test according to JIS R 1607. The veneer porcelains for the zirconia all-ceramic crown were fired on two different thicknesses (0.4 mm and 0.8 mm) of zirconia frames (ZAC). As the controls, veneer porcelains fired on metal frames for porcelain fused to metal crown (PFM) were used. The fracture toughness value evaluated by measuring the extent of cracking associated with a Vickers indentation on the veneer porcelain part was significantly larger at the points of 1.5 mm in 0.4 mm thickness frame of ZAC samples compared with in the 0.8 mm frame.

Key words. Fracture toughness, Veneer porcelain, Vickers indentation, Y-TZP, Zirconia all-ceramic

1 Strength of Veneer Porcelain on Zirconia Frame

Yttria-stabilized tetragonal zirconia (Y-TZP) frameworks for all-ceramic restorations have been increasingly used for crowns and fixed partial dentures. Since the Y-TZP framework provides sufficient mechanical strength, these restorations are

M. Kudo (✉), S. Miura, R. Inagaki, and M. Yoda
Division of Fixed Prosthodontics, Graduate School of Dentistry, Tohoku University,
4-1 Seiryō-Machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: m-kudo@dent.tohoku.ac.jp

M. Kikuchi
Division of Dental Biomaterials, Tohoku University Graduate School
of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai, Japan

J. Cho and K. Sasaki
Division of Advanced Prosthetic Dentistry, Graduate School of Dentistry,
Tohoku University, 4-1 Seiryō-machi, Aoba-ku, Sendai, Japan

applied to not only anterior regions but also posterior regions. Because of aesthetic demands, the veneer porcelains have to be fired on these frameworks. Recently, chipping of the veneer porcelain has been recognized as a clinical problem with zirconia all-ceramic restoration [1, 2]. The zirconia thickness is thought to be the major determinant for the strength of veneer porcelain.

2 Evaluation of the Strength of Veneer Porcelain

The strength of the veneer porcelain could be evaluated with a fracture toughness test according to JIS R 1607. The value was calculated by measuring the extent of cracking associated with a Vickers indentation on the veneer porcelain part of the cross-section.

As the test samples, the veneer porcelain for the zirconia all-ceramic crown was fired on two different thicknesses of zirconia frames (10 mm×15 mm×0.4 mm; ZAC (0.4), 10 mm×15 mm×0.8 mm; ZAC (0.8)). After the veneer porcelain was fired, the total thickness of the entire veneer porcelain was 3.0 mm. The veneer porcelain samples fired on gold alloy (KIK, ISHIFUKU, Japan) frames for porcelain fused to a metal crown (PFM) were used as the control, Veneer porcelain samples fired without a zirconia frame (ZAC(0)) were also evaluated. For each frame, the porcelain firing procedure was carried out according to the manufacturer's instructions. After firing, each sample was cut perpendicular to the flames, and the cross-sectional surface was ground.

The measurements were conducted at different distance points from the frame (0.5 mm, 1.0 mm, 1.5 mm, and 2.0 mm; total 24 points). The data were analyzed by two-way ANOVA followed by Tukey's test ($\alpha=0.05$) to identify any significant difference in the frame thickness and the distance from the frame.

3 Fracture Toughness of Veneer Porcelain Strength Related to the Thickness of Veneer Porcelain

The fracture toughness values of the veneer porcelain of ZAC (0.4) and ZAC (0.8) were no significantly different. However, the toughness of the veneer porcelain of ZAC (0) was significantly larger than ZAC (0.8). The fracture toughness value of the veneer porcelain at the points of 1.5 mm in ZAC (0.4) was significantly larger than those in the 0.8 mm frame. Compared with ZAC samples, the fracture toughness value of the veneer porcelain of PFM samples were about 1.5 times larger, although there were no significant differences between PFM (0.4) and PFM (0.8).

4 Conclusion

The results of our experiment suggested that there was an effect of zirconia frame thickness on the fracture toughness of veneer porcelain for zirconia all-ceramic restoration. However, it is considered that the factor of chipping causes lower fracture toughness for zirconia all-ceramic restoration.

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Long-Term Clinical Evaluation of Cervical Composite Resin Restorations Treated with the Self-Etch Bonding System

Hiromi Sasazaki, Hideki Sato, Tatsuo Endo,
and Masashi Komatsu

Abstract. The purpose of this study was to evaluate the long-term periodic changes of marginal adaptation of cervical composite resin restorations. Class V saucer type cavities or cervical cavities were prepared on the labial or buccal surfaces of vital human incisors, canines, premolars and molars. A total of 124 restorations were placed to 40 patients (13 males and 27 females) by one operator. Two self-etch bonding systems were used in this study. Clinical findings of these fillings were periodically observed. In order to observe the marginal adaptation, impressions of these restorations were taken and precision replicas were made. These replicas were observed by scanning electron microscope. In the Fluoro bond group (Shofu, Kyoto, Japan) and Mega bond group (Kuraray, Okayama, Japan), maximum observation period is 4,582 days. In many cases of enamel and enamel dentin margin cavities, marginal steps were observed after 1 year. The widths of steps were extended with time. Three dislodged cases were observed in Fluoro bond group and two dislodged cases were observed in Mega bond group during 10 years.

Key words. Clinical performance, Marginal adaptation, Replica, Self-etch bonding system, SEM

1 Introduction

Self-etch bonding system was now widely used in the world. But it is reported that the bonding strength to the enamel of these materials was weaker than phosphoric acid etching. We reported in the previous study, when the bonding layer was exposed

H. Sasazaki (✉), H. Sato, T. Endo, and M. Komatsu
Division of Operative Dentistry, Department of Restorative Dentistry,
Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku,
Sendai 980-8575, Japan
e-mail: sasazaki@ddh.tohoku.ac.jp

at the margin, it was abraded and marginal step was clearly observed in an early stage. If marginal steps were formed, abrasion and microfracture of composite resin progressed with time [1]. The purpose of this study was to evaluate the long-term periodic changes of marginal adaptation of cervical composite resin restorations.

2 Materials and Methods

Under local anesthesia or without an anesthesia, class V saucer type cavities or cervical cavities were prepared on the labial or buccal surfaces of vital human incisors, canines, premolars and molars. A total of 124 restorations were placed to 40 patients (13 males and 27 females) by one operator. Fluoro bond and Mega bond were applied according to the manufacturer's instructions. Light-cured type composite resins (Lite fil II A: Shofu, Kyoto, Japan, Clearfil AP-X: Kuraray, Okayama, Japan) were inserted into these cavities and polymerized. Clinical findings of these fillings were periodically observed. In order to observe the marginal adaptation, impressions of these restorations were taken and precision replicas were made. These replicas were observed by scanning electron microscope.

3 Results

In the Fluoro bond group, Mega bond group, maximum observation period is 4,582 days. Spontaneous pain, cold water pain, warm water pain, percussion pain, occlusal pain and gingival damage were not detected. In many cases of enamel and enamel dentin margin cavities, marginal steps were observed after 1 year. In the Fluoro bond (+phosphoric acid), marginal adaptation of early stage was better than Fluoro bond (–phosphoric acid) group. The widths of steps were extended with time. Three dislodged cases were observed in Fluoro bond group and two dislodged cases were observed in Mega bond group during 10 years.

4 Discussion and Conclusion

When the bonding layer was exposed at the margin, abrasion progressed from the exposed bonding layer and then marginal fracture of resin materials occurred. Enamel etching effectively improved the adaptation to enamel.

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Evaluation of Metal Allergies Using the Patch Test in Patients with Skin or Oral Mucosal Diseases

Hideki Sato, Hiromi Sasazaki, and Masashi Komatsu

Abstract. Allergy patients, including those with skin or oral mucosal diseases, are said to have a significantly higher rate of positive reactions in skin patch testing when compared to healthy controls. The purpose of this study was to analyze the pattern of positive skin reactions to metal salts in patients with eczema, skin eruptions, contact dermatitis, pustulosis palmaris et plantaris, diseases of the oral mucosa or tongue, atopic dermatitis and allergic diathesis. This study documented that Ni and Pd have the highest allergenic potency, followed by Zn, Sn, Co, Cr, Pt, Hg, and Au. Patients with contact dermatitis, pustulosis palmaris et plantaris and allergic diathesis had an especially high incidence of positive patch test results in response to Ni, while patients with contact dermatitis and lichen planus showed high frequency of positive reactions to Pd, and patients with atopic dermatitis showed high rates of positive skin reactions to Co.

Key words. Atopic dermatitis, Contact dermatitis, Lichen planus, Metal allergy, Pustulosis palmaris et plantaris

1 Introduction

Patch testing is a way of identifying whether a substance that comes in contact with the skin is causing inflammation of the skin or oral mucosa. Although patch testing is not very precise, it is however very simple and does not involve the use of needles.

H. Sato (✉), H. Sasazaki, and M. Komatsu
Division of Operative Dentistry, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
and
Division of Dental Biomaterials, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: yamasa@m.tohoku.ac.jp

Medical devices (artificial joints, needles and pacemakers), pierced earrings, necklaces and dental metallic restorations or appliances may cause metal allergies.

2 Materials and Methods

In this study, patch testing of metal salts was performed on 200 patients (56 males, 144 females) with eczema, skin eruptions, contact dermatitis, pustulosis palmaris et plantaris, diseases of the oral mucosa or tongue, atopic dermatitis and allergic diathesis treated at Tohoku University Hospital in the last 5 years. The metal salts were mixed with a non-allergic material (base) to a suitable concentration. They were then placed in direct contact with the skin, on the forearm, with small gauzes. Adhesive tape was used to fix them in place, and the test sites were marked with a felt-tip pen. Adhesive tape, reagents (Torii Pharmaceutical Co., Tokyo, Japan) and commercially pure titanium powder (Ti: >99.7%, O: 0.04%, Fe: 0.03%; Toho Titanium Co., Kanagawa, Japan) were used. Patch testing was done on a skin site without apparent dermatitis. The patches were left in place for 48 h, during which time patients were advised to not wash the area, sweat excessively, or play vigorous sports. Patients were also advised to not expose the patches to sunlight or other sources of ultraviolet light. After 48 h the patches were removed by the patient. Once the tape had been removed, the patients could bathe normally but were advised to avoid scrubbing the forearm in order to prevent removal of the markings. An initial reading was performed after 48 h, and additional readings were performed at 72 and 168 h after initial placement. A positive reaction was defined as redness, mild swelling or formation of a small blister.

3 Results and Discussion

Ni and Pd evoked the highest number of positive reactions in the entire study sample, with 17.9% and 19.6% of males and 28.5% and 25.7% of females showing positive reactions, respectively. Patients with the following diseases showed a remarkably high rate of positive skin reactions to metal salts: pustulosis palmaris et plantaris: Ni 35.3%; contact dermatitis: Ni 48.1%, Pd 40.7%; lichen planus: Pd 33.3%; allergic diathesis: Ni 34.8%, Pd 30.4%, Sn 30.4%; atopic dermatitis: Co 33.3%. A majority of males (32/56, 57.1%) and females (92/144, 63.9%) had some general reaction to metal salts.

Sterilization Effect in Low-Pressure Discharge Plasma Using Non-toxic Gas

Kaoru Tamazawa, Yoshinori Tamazawa, and Hidetoshi Shimauchi

Abstract. The purpose of this study is to evaluate the efficacy of sterilization by plasma discharge using non-toxic gas. One hundred and eight samples inoculated with 10^5 spores (*Geobacillus stearothermophilus*, ATCC#7953, Namsa) were divided into three groups of thirty-six each by non-toxic gas (O_2 , O_2/H_2O , $O_2/N_2/H_2O$). These samples were treated in plasma reactor (13.56 MHz, PACK-3®, YAC) for 10 min at 60°C by varying gas pressure and gas flow, and were incubated in trypticase soy broth (BBL) at 58°C for 72 h. O_2/H_2O plasma group was significantly higher (chi-square test, $p < 0.05$) than two other groups in negative-culture rate, and samples treated at 13 Pa showed all negative-culture. It is speculated that H_2O is expected to generate OH and OH_2 radicals to increase the sterilization effect. O_2/H_2O plasma treated at 13 Pa showed high sterilization effect in low temperature for short time.

Key words. Non-toxic gas, Oxygen, Plasma, Spore, Sterilization

1 Introduction

A new low-temperature sterilization technique to replace ethylene oxide gas sterilization has been needed [1]. Currently research applied plasma technology has been extended to microbiological field. The purpose of this study is to evaluate the efficacy of sterilization by plasma technology using non-toxic gas.

K. Tamazawa (✉) and H. Shimauchi

Division of Periodontology and Endodontology, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: tamazawa@dent.tohoku.ac.jp

Y. Tamazawa

Division of Infection Control, Tohoku University Hospital, Sendai, Japan

2 Materials and Methods

10^5 *Geobacillus stearothermophilus* spores (ATCC#7953, suspension type, Namsa), were inoculated on the stainless steel dish (Φ 10 mm, depth = 1 mm) as spore sample. One hundred and eight samples were divided into three groups of thirty-six by gas composition (O_2 , O_2/H_2O , $O_2/N_2/H_2O$), and were treated in plasma reactor (13.56 MHz, PACK-3[®], YAC) under the condition of fixed output (150 W), chamber temperature (60°C), and discharge time (600 s). Sterilization effect were studied by varying gas composition, gas pressure (13–100 Pa), and gas flows (50–500 sccm: standard cm³/min). Separate from these, twenty-eight samples were treated by varying temperatures (60–140°C) without plasma discharge.

All these samples after plasma treatment were incubated in trypticase soy broth (BBL) at 58°C to evaluate the efficacy of sterilization. Bacterial growth after 72 h incubation was recorded as positive or negative by visual inspection of turbidity.

3 Results

Negative culture rate of O_2/H_2O group was significant higher (chi-square test, $p < 0.05$) than that of two other groups. Besides the samples treated at 13 Pa were all negative culture. Namely, O_2/H_2O plasma at 13 Pa showed remarkably effective, decreasing the surviving spore density by less than 5 logs. However, in the samples without plasma discharge, the temperature required to obtain a negative culture was at more than 120°C under low pressure, and was at more than 140°C under atmosphere pressure.

4 Discussions

O_2/H_2O mixture plasma showed high sterilization effect. It is speculated that H_2O is expected to generate OH and OH_2 radicals [2]. The parameter of gas composition, gas pressure and gas flow are remarkably important since strongly affect the rate of generating reactive species to increase sterilization effect. If the more optimal combination of these parameters is found out, the higher sterilization effect will be achieved, without toxic gas such as hydrogen peroxide gas used in hydrogen peroxide plasma sterilization system (Sterrad[®], J & J).

Acknowledgment This study was supported by the JSPS grant (No. 18390503).

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Tarnish Resistance of Experimental Ti–Ag Alloys in 0.1 % Na₂S Solution

Masatoshi Takahashi, Masafumi Kikuchi, and Yukyo Takada

Abstract. We developed experimental Ti–Ag alloys and characterized their properties. The mechanical properties and machinability of the Ti–Ag alloys were superior to those of pure titanium. The Ti–Ag alloy surfaces inhibited biofilm formation by *Streptococcus mutans*. The spontaneous formation of calcium phosphates on the Ti–Ag alloys upon immersion in simulated body fluid was confirmed. The corrosion resistance of the Ti–Ag alloys in a 0.9% NaCl solution and a 1% lactic acid solution was comparable to that of pure titanium. Further, we performed a tarnish resistance test, and found that the Ti–Ag alloys had excellent tarnish resistance in 0.1% Na₂S solution, comparable or superior to that of pure titanium.

Key words. Biomaterial, Dental alloy, Tarnish resistance, Ti–Ag alloy

1 Introduction

As part of our studies toward the development of new dental titanium alloys with better machinability and mechanical properties than unalloyed titanium, we developed experimental Ti–Ag alloys. The strength and hardness of the Ti–Ag alloys increased with the concentration of Ag [1]. The machinability of the Ti–Ag alloys was superior to that of pure titanium [1]. We also found that the Ti–Ag alloy surfaces inhibited *Streptococcus mutans* biofilm formation, but pure titanium surfaces did not [2]. Moreover, calcium phosphates spontaneously formed on the Ti–Ag alloy surfaces when immersed in simulated body fluid [3]. These results suggest that the Ti–Ag alloys are suitable for use in dental prostheses and implants.

M. Takahashi (✉), M. Kikuchi, and Y. Takada
Division of Dental Biomaterials, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: takahashi@m.tohoku.ac.jp

2 Tarnish Resistance

Dental alloys should have good corrosion resistance and sufficient tarnish resistance, since they are used in the human mouth. Electrochemical behavior and the ions released from the Ti–Ag alloys in a 0.9% NaCl solution and a 1% lactic acid solution were investigated in our previous study [4, 5]. Ti–Ag alloys with up to 25% Ag have good corrosion resistance for dental alloys [4, 5]. However, they may suffer sulfidation because of the presence of Ag.

Ingots of Ti–Ag alloys with 20 and 25 mass% Ag and pure titanium were prepared in an argon-arc melting furnace and cast into plate specimens. Pure silver specimens were also prepared. The polished specimens were immersed in a 0.1% Na₂S solution at 37°C for 72 h. Immersed and nonimmersed specimens were visually compared. Color differences (ΔE^*_{ab}) between specimens before and after immersion were determined by a color-difference meter (CR-400, Konica Minolta). The data were statistically analyzed.

There were no visible color changes between immersed and nonimmersed specimens except in the case of the pure silver specimen. The color differences (ΔE^*_{ab}) of Ti–Ag alloys tended to decrease as the concentration of Ag increased, although there was no significant difference from that of pure titanium. The ΔE^*_{ab} for pure silver was significantly higher than for other metals.

3 Conclusions

The Ti–Ag alloys had excellent tarnish resistance in Na₂S solution, comparable or superior to that of pure titanium.

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The Development of a Dental Unit Without a Water Supply Pipe to Prevent Water Contamination

Yoshinori Tamazawa and Kaoru Tamazawa

Abstract. Water contamination from conventional dental units is serious problem. The ADA (American Dental Association) set recommended standard level of water quality from dental units as 200 CFU/mL (heterotrophic bacteria). However, there are no reports on dental units used worldwide meeting this standards. The purpose of this study was to develop a units without a water supply pipe, and meet water quality standards set by the ADA.

So, we developed a dental unit installing a built-in removable water supply tank, without a water supply pipe to eliminate biofilm, the source of water contamination in the pipe. And, we grew heterotrophic bacteria on R2A agar medium at 20°C for 7 days, and measured bacterial numbers for water quality tests. The results showed that the number of heterotrophic bacteria from turbine, engine-handpiece and three-way syringe ranged between 2 and 80 CFU/mL, achieving the standard value of the ADA.

Key words. Bacteria, Biofilm, Dental unit, Infection control, Water contamination, Water supply pipe

1 Introduction

Water contamination from conventional dental units is serious problem. The ADA set recommended standard level of water quality from dental units as 200 CFU/mL (heterotrophic bacteria) [1, 2]. However, there are no reports on dental units used

Y. Tamazawa (✉)

Dental Clean Room, Tohoku University Hospital, 4-1 Seiryō-machi,
Aoba-ku, Sendai 980-8575, Japan
e-mail: y-tama@dent.tohoku.ac.jp

K. Tamazawa

Division of Periodontology and Endodontology, Tohoku University Graduate School
of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

worldwide meeting this standards. The purpose of this study was to develop a units without a water supply pipe, and meet water quality standards set by the ADA.

2 Materials and Methods

We removed the water supply pipe from a dental unit, installing a built-in removable water supply pipe tank. Firstly, we investigated the tank shape, size and the tank set-up. Secondly, we investigated the water volume control control system. Finally, we grew heterotrophic bacteria on R2A agar at 20°C for 7 days, and measured bacterial numbers.

3 Results and Discussion

1. Development of a dental unit with removable built-in tank: We removed the water supply pipe, and installed a removable built-in tank (2,000 mL).
2. Water volume control system: The JIS standard value of a dental unit water flow rate (50 mL/min under 0.2 MPa) was achieved when turbine, engine-handpiece and three-way syringe were operated.
3. Bacterial test: The number of heterotrophic bacteria in the conventional type and the tank type was tested. The results showed that the number of bacteria from the turbine, engine-handpiece and three-way syringe ranged between 100 and 4,300 CFU/mL in the conventional type. We operated the tank type for 5 consecutive days, and counted bacteria at 6 months and 1 year after dental unit completion. The results ranged from 2 to 80 CFU/mL, achieving the standard value of the ADA.

4 Discussion

The conventional type has a water supply pipe 6–7 m long. In contrast, the pipe of the tank type we developed was 26 cm. The water supply pipe where biofilm develops is almost completely removed.

5 Conclusions

The dental unit we developed with a removable built-in water tank met the ADA standards for water quality.

Acknowledgment This study was supported by the JSPS grant (No.19390489).

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Macrophage Reaction Against Sub-micron Titanium Particles

Masayuki Taira, Minoru Sasaki, and Shigenobu Kimura

Abstract. Around titanium (Ti) implants, peri-implantitis is sometimes found. This study clarified that macrophage actively phagocytized and intra-cellulary refined sub-micron Ti particles (simulated wear debris) while producing abundant inflammatory cytokines. The amounts of inflammatory cytokines were significantly increased by concomitant stimulation with Ti particles and lipopolysaccharide (LPS).

Key words. Titanium, Sub-micron particles, Macrophage, Phagocytosis, Inflammatory cytokines

Titanium (Ti) wear debris often causes osteolysis around Ti implants and artificial joints. The purpose of this study was (1) to morphologically observe, (2) to evaluate inflammatory cytokine productions and (3) to examine gene expressions of human macrophages (PMA-differentiated THP-1 cells) phagocytizing sub-micron Ti particles.

1. SEM and TEM observations revealed that the 24 h cultured macrophages actively phagocytized sub-micron Ti particles and accumulated them in intracellular phagosomes in which refinement of Ti particles took place (Fig. 1) [1].
2. The macrophages were also cultured for 24 h in four media with and without LPS and sub-micron Ti particles; and the contents of inflammatory cytokines (TNF-alpha, IL-1beta and IL-6) in the culture supernatants as well as cell viabilities were measured. While cell viabilities in four media remained constant, not

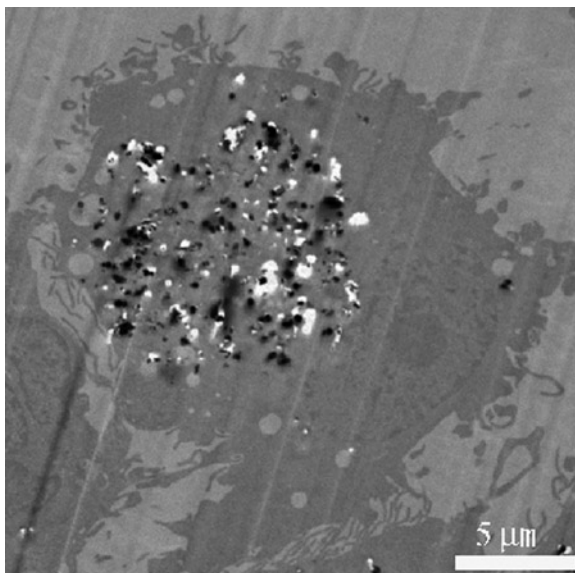
M. Taira (✉)

Department of Biomedical Engineering, Iwate Medical University,
Nishi-Tokuda 2-1-1, Yahaba-chou, Shiwa-gun, Iwate 028-3694, Japan
e-mail: mtaira@iwate-med.ac.jp

M. Sasaki and S. Kimura

Division of Molecular Microbiology, Department of Microbiology, Iwate Medical University,
Nishi-Tokuda 2-1-1, Yahaba-chou, Shiwa-gun, Iwate 028-3694, Japan

Fig. 1. TEM observation of 70 nm thick macrophage phagocytizing sub-micron Ti particles. Macrophage accumulated sub-micron Ti particles in intracellular phagosomes in which refinement of Ti particles took place



only LPS activation but also phagocytosis of sub-micron Ti particles caused the macrophages to abundantly produce the three inflammatory cytokines. Dual stimulation of LPS and sub-micron Ti particles synergistically increased productions of these cytokines [1].

3. Microarray analyses with DNA allergy chip with 205 gene spots clarified that phagocytosis of sub-micron Ti particles and LPS independently and synergistically up-regulated 17 inflammation-related genes more than two-fold. The extensive expressions of three genes (IL-1 β , IL-6 and IL-8) were further confirmed by real-time quantitative PCR. It turned out that dual stimulation of LPS and sub-micron Ti particles most up-regulated these three genes, followed by LPS while sub-micron Ti particles moderately but least increased [2].

It was concluded that wear and LPS should be avoided around Ti implants.

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The Influence of Surface Treatment of Acrylic Denture Base Resin on Peel Bond Strength Between Resilient Denture Liners

WeiQi Wang, Guang Hong, Maimaitishawuti Dilinuer,
Taizo Hamada, and Keiichi Sasaki

Abstract. This study aimed to influence of surface treatment of acrylic denture base resin on peel bond strength between resilient denture liners. The results suggest that the surface treatment of denture base resin only influences the peel bond strength between autopolymerized resilient denture liners.

Key words. Peel bond strength, Resilient denture liner, Surface treatment

1 Background

The loss of bond of a resilient denture liner (RDL) to a denture base resin is a common cause of failure in denture lining. Bond strength is one of the most important factors which decide the use period of the RDL as well as the material's own durability. The purpose of this study was to determine the influence of surface treatment of acrylic denture base resin on peel bond strength between RDL.

W. Wang, M. Dilinuer, and K. Sasaki
Division of Advanced Prosthetic Dentistry, Graduate School of Dentistry,
Tohoku University, Sendai, Japan

G. Hong (✉)
Liaison Center for Innovative Dentistry, Graduate School of Dentistry,
Tohoku University, Sendai, Japan
e-mail: hong@m.tohoku.ac.jp

T. Hamada
Department of Oral Health Care Promotion, Graduate School of Dentistry,
Tohoku University, Sendai, Japan

2 Material and Methods

In this study, 1 heat-polymerized (HP) silicone type RDL (Molloplast-B), 2 HP acrylic type RDL (Super-Soft, Vertex-Soft), 4 autopolymerized (AP) silicone type RDL (UfiGel-C, Sofreliner-Tough, Sofreliner-Soft, Reline-UltraSoft), and 1 AP acrylic type RDL (COE-Soft) were used. The acrylic denture base resin (Acron) were polymerized according to the manufacturer's recommendation to the rectangular blocks. The section surfaces of peel test specimens were treated with 200 (L-Treat), 400 (M-Treat) and 800 (H-Treat) grit waterproof abrasive paper. The mixed resilient denture liner was placed on the surface of the test blocks, and nine specimens were prepared for each material and treatment. Specimens consisted of a 75×25×3 mm denture base resin plate and a 75×25×2 mm resilient denture liner which were bonded over 25 mm of the length of the samples and separated over the remaining 50 mm. The peel bond strengths (Ps) were measured with universal testing machine (Model-5565). The modes of debonding were characterized as tear and peel, or snap. Statistical analysis was performed using 1-way analysis of variance (ANOVA), Student–Newman–Keuls multiple comparison tests (S–N–K test) ($\alpha=0.05$).

3 Results and Discussion

The Fig. 1 shows the peel bond strength of each materials and treatments. A significant difference ($p<0.05$, ANOVA) was found between the resilient denture liners for peel bond strength. In the L-Treat group, heat-polymerized acrylic material VSN

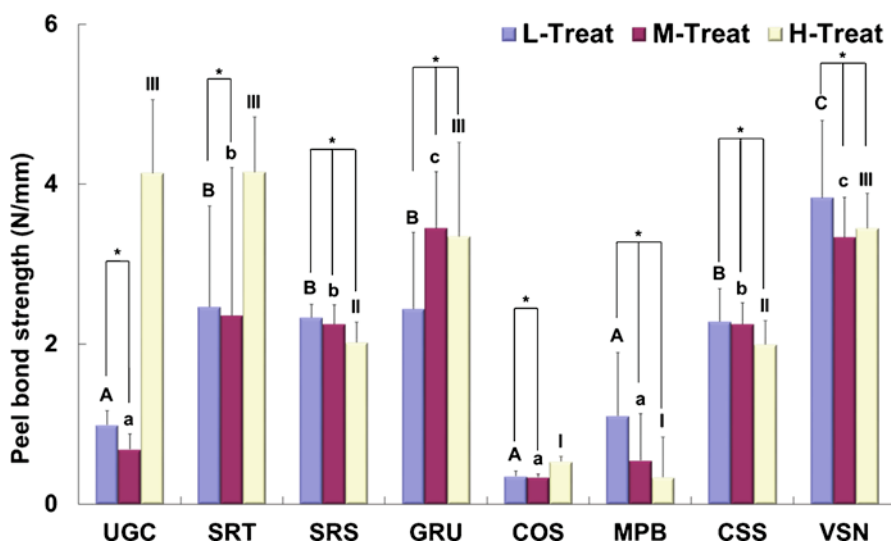


Fig. 1. Peel bond strength of each materials and treatments. Identical letters indicate no significant differences, asterisks indicate significant differences, $p>0.05$, SNK test

Table 1. Debonding mode of each materials and treatments

	L-Treat			M-Treat			H-Treat		
	Tear	Peel	Snap	Tear	Peel	Snap	Tear	Peel	Snap
UGC	0	9	0	0	9	0	0	5	4
SRT	0	6	3	0	5	4	0	0	9
SRS	6	0	3	0	0	9	0	0	9
GRU	0	8	1	0	3	6	0	3	6
COS	0	0	9	0	0	9	0	0	9
MPB	0	3	6	0	6	3	1	7	1
CSS	0	0	9	0	0	9	0	0	9
VSN	0	0	9	0	1	8	0	4	5

showed highest value of peel bond strength than other materials. In the M-Treat group, GRU and VSN showed significantly higher values. In the H-treat group, UGC, SRT, GRU and VSN showed the higher value of peel bond strength than other materials. COS and MPB showed significantly lower values than other materials in all treatment groups.

The peel bond strength of H-Treat samples excepted SRS and GRU showed highest value than samples of L-Treat and M-Treat ($p < 0.05$, SNK-test). No significant difference ($p > 0.05$, ANOVA) was found between the different surface treatment for peel bond strength of heat-polymerized resilient denture liners.

The Table 1 shows the debonding mode of each materials and treatments. All samples of COS and CSS showed Snap mode in all treatment groups. The peel mode of remaining samples excepted VSN significantly increased with rough treatment. The sample of VSN showed the opposite results. Tear mode was showed only L-Treat group of SRS and H-Treat group of MPB.

4 Conclusions

Within the limitations of this study, the results show that the surface treatment of denture base resin only influences the peel bond strength between autopolymerized resilient denture liners.

Session IV

Social Interface

Socioeconomic Inequalities in Tooth Loss Among Japanese

Kanade Ito, Jun Aida, Shintaro Wakaguri, Kenji Takeuchi,
Yuki Noguchi, and Ken Osaka

Abstract. Few studies have examined the association of multiple indices of socioeconomic status on tooth loss. The aim of this study was to investigate the association between multiple indices of socioeconomic status and tooth loss among Japanese. We conducted a web-based survey in 2010. A total of 4,989 people aged 20–69 years participated in our study. Cross tabulation and logistic regression analysis were used to determine the association between having missing teeth and socioeconomic status. A multiple logistic regression analysis showed that respondents with the lowest educational attainment had a 2.05 times higher odds ratio (95%CI:1.36–3.10) for having missing teeth compared to those with highest education. Similarly, respondents with the lowest house-hold income had a 1.25 times higher odds ratio (95%CI:1.01–1.55) for having missing teeth compared to those of highest group. Educational attainment and house-hold income were significantly associated with socioeconomic inequalities in tooth loss.

Key words. Socioeconomic status, Tooth loss

K. Ito (✉), J. Aida, S. Wakaguri, K. Takeuchi, and K. Osaka
Department of International and Community Oral Health,
Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: kanade92@gmail.com

Y. Noguchi
Department of International and Community Oral Health,
Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi,
Aoba-ku, Sendai 980-8575, Japan
and
Division of Oral Health Sciences, Department of Health Sciences School
of Health and Social Services, Saitama Prefectural University,
820 Sannomiya, Koshigaya, Saitama 343-8540, Japan

1 Introduction

Several studies have suggested the association between socioeconomic status and tooth loss. Educational attainment, occupation and house-hold income were frequently used as socioeconomic indices [1–3]. However few studies have examined the association of multiple indices of socioeconomic status on tooth loss especially in Japan. The aim of this study was to investigate the association between multiple indices of socioeconomic status and tooth loss among Japanese.

2 Methods

We conducted a web-based survey during April to November 2010. From the registered men and women aged between 20 and 69 years old in the web research agency, a total of 4,989 people participated in our study. Our questionnaire included educational attainment, occupation and house-hold income as socioeconomic indices. The outcome variable we used was having missing teeth or not. Explanatory variables were educational attainment, occupation and house-hold income. Cross tabulation and logistic regression analysis were used to determine the association between having missing teeth and socioeconomic status. Sex and age were used as covariates.

3 Results

According to chi-square test, the distribution of respondents with missing teeth was significantly differed by sex, age, educational attainment and occupation. A multiple logistic regression analysis showed that respondents with the lowest educational attainment had a 2.05 times higher odds ratio (95%CI:1.36–3.10) for having missing teeth compared to those with highest education. Similarly, respondents with the lowest house-hold income had a 1.25 times higher odds ratio (95%CI:1.01–1.55) for having missing teeth compared to those of highest group.

4 Conclusion

Educational attainment and house-hold income, but not occupation were significantly associated with socioeconomic inequalities in tooth loss even after being adjusted for sex, age and each socioeconomic variable.

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Gender Differences in the Association Between Self-Rated Oral Health and Socioeconomic Status Among Japanese

Shintaro Wakaguri, Kanade Ito, Jun Aida, Kenji Takeuchi,
and Ken Osaka

Abstract. We investigated the association between self-rated oral health and socioeconomic status, stratified by gender. There were partially different between male and female.

Key words. Gender difference, Self-rated oral health, Socioeconomic status

1 Introduction

Health inequalities caused by various social determinants have been reported. There are the gender differences in health inequalities relating socioeconomic status [1]. We should concern the gender difference to investigate the association between health and socioeconomic factors. Self-ratings of health are robust predictors of subsequent health outcomes, such as functional ability, morbidity and mortality [2–4]. Self-rated oral health (SROH) is also reported to be a simple direct way of capturing perception of oral health [5]. The aim of this study was to investigate the gender differences in the association between SROH and socioeconomic status.

S. Wakaguri (✉), K. Ito, J. Aida, K. Takeuchi, and K. Osaka
Department of International and Community Oral Health,
Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi,
Aoba-ku, Sendai 980-8575, Japan
e-mail: a8dd5044@s.tohoku.ac.jp

2 Methods

2.1 Study Sample

We conducted a web-based survey during April to November 2010. A total of 4,989 people participated in our study from the registered men and women aged between 20 and 69 years old in the web research agency. Because employment status was a major predictor of our study, students were excluded from the analysis. Then, 3,876 persons were included into the analyses.

2.2 Data Collection

We obtained data by self-administered questionnaire on website. The questionnaire included the information on socioeconomic variables, employment status of respondent, occupation of householder, educational attainment, going vacation trips in the past one year, in possession of bedroom and number of own cars and monthly household income. The SROH was rated as follows: 'excellent', 'good', 'poor' or 'very poor'. In analyzing the data we categorized into 'good' (excellent/good) and 'poor' (poor/very poor).

2.3 Data Analysis

The univariate associations between SROH and each socioeconomic factor were evaluated by the cross-tabulations and chi-squared tests. A multiple logistic regression model was used to estimate odds ratios for poor SROH. In the analyses, age and socioeconomic variables which were significant in the univariate analyses were used as variables. All analyses were stratified by gender.

3 Results

The numbers of male and female participants were 1,907 (49.2%) and 1,969 (50.8%), respectively. The participants with good SROH were 1,042 (54.6%) in males and 1,142 (58.0%) in females. The univariate analyses showed significant associations between SROH and going vacation trips and household income among male. Among female, educational attainment, going vacation trips and household income showed significant association with SROH. After adjusting age, household income in both male and female, educational attainment and going vacation trips in female showed significant association with SROH. Among male, the logistic

regression showed that compared to the participants with income of ¥500,000 or more, participants with lower income showed significantly higher odds ratios for poor SROH [$<¥200,000$: 1.74 (95% CI: 1.27–2.39), ¥200,000–299,999: 1.47 (95% CI: 1.13–1.93), ¥300,000–399,999: 1.42 (95% CI: 1.08–1.86)]. Among female, compared to the participants with income of ¥500,000 or more, participants with the lowest income showed 1.46 times higher odds ratios for poor SROH (95% CI: 1.08–1.96). In addition, compared to the female participants with the highest education attainment (college or university degree), participants with high school degree showed 1.67 times higher odds ratios for poor SROH (95% CI: 1.31–2.12). Female participants without vacation trip in the past 1 year showed 1.23 times higher odds ratios compared to the participant with vacation trips (95% CI: 1.01–1.50).

4 Conclusion

There seem some gender differences in the association between the SROH and socioeconomic status.

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Collaboration of Medical and Dental Facilities Promotes Oral Health Care in Hospitals

Jun Harako, Toru Tamahara, Ryoichi Hosokawa, Kazunori Kimura, Moritoshi Komagata, Koji Hanaoka, Seiichi Aonuma, Fusako Okuya, Kaoru Itagaki, Yukiko Akai, Kazuhiko Tamura, Hidetoshi Sonobe, and Takeyoshi Koseki

Abstract. Oral health care is important for the acute hospitalized patients, to avoid and reduce negative effects or discomfort during the intensive medical care and burdensome medical treatment. It was reported by a questionnaire survey that oral health care was performed by nurses and careworkers in almost all hospitals in Sendai city, except some specialized functional facilities. Also, the collaborations with the dental offices were conducted in about two thirds of the hospitals. The major problems which interfered with the oral health care in hospitals were serious shortage of manpower, inadequate medical and technical service payment, and the lack of experienced stuffs. These problems might be caused by the lack of information and supervision from the dental professions on site in hospitals. To promote and

J. Harako, T. Tamahara, and R. Hosokawa
Division of Preventive Dentistry, Department of Oral Health and Development Sciences,
Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi,
Aoba-ku, Sendai, Japan

K. Kimura, M. Komagata, K. Hanaoka, S. Aonuma, F. Okuya, K. Itagaki, Y. Akai,
K. Tamura, and H. Sonobe
Division of Oral and Dental Health Care, Council for Community
Healthcare of Sendai, Sendai, Japan

T. Koseki (✉)
Division of Preventive Dentistry, Department of Oral Health and Development Sciences,
Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi,
Aoba-ku, Sendai, Japan
and
Division of Oral and Dental Health Care, Council for Community
Healthcare of Sendai, Sendai, Japan
and
Community Oral Health Promotion Office, Tohoku University Graduate
School of Dentistry, Sendai, Japan
e-mail: yobou@dent.tohoku.ac.jp

reinforce the oral health care in hospitals, further close and community-based collaboration between the hospitals and the neighboring dental clinics are necessary.

Key words. Collaboration, Hospital, Hospitalized patients, Oral health care

1 Introduction

Oral health care is important for the acute hospitalized patients, to avoid and reduce negative effects or discomfort during the intensive medical care and burdensome medical treatment. To promote the sufficient oral health care for these patients, it is important to establish the vigorous collaboration and the close partnership between the medical and dental facilities. In this paper, we discuss the current status of collaboration on oral health care in community medicine of Sendai city, based upon the questionnaire survey of the hospitals.

2 Current Status of Oral Health Care in Hospitals

The questionnaires, which were answered by more than half of the hospitals in Sendai city, reported that oral health care was performed by nurses and careworkers in almost all hospitals in Sendai city, except some specialized functional facilities such as dialyzed treatment center. Also, the collaborations with the dental offices were conducted in about two thirds of the hospitals. These oral health care were included with oral hygiene, cleaning their denture, and mouth rinsing. The hospitals constituted with dental clinics instructed more use of interdental cleaning devices and moisturizing agents. Oral rehabilitation for eating, swallowing, speech, and physical exercise were provided by the nurses, speech therapists and medical doctors. Three quarters of the hospitals recognized the needs of continuous skill-up learning of their oral health care. These indicated that oral health care are commonly provided in hospitals in Sendai city.

3 Collaboration of Hospital and Dental Office for Promotion of Oral Health Care

Collaboration of hospitals and dental offices were reported by two thirds of the hospitals, and by four fifths of the hospitals in which dental hygienists were employed. The major problems which interfered with the oral health care in hospitals were serious shortage of manpower, inadequate medical and technical service payment, and the lack of experienced stuffs. In details, first and second problems were more complained by the hospitals which constitute with dental clinics, and third problem was more complained by the hospitals without dental clinics.

4 To Promote and Enforce Oral Health Care in Hospitals

Almost all hospitals provide oral health care for the hospitalized patients, however, there is some problems arresting the promotion of oral health care. These problems may be caused by the lack of information and supervision from the dental professions on site in hospitals. It is well-known that the oral health care of hospitalized patients prevents the side-effects of medical treatments, thus, promotion of oral health care of the patients brings the higher quality of daily life in fighting against the diseases. To promote and reinforce the oral health care in hospitals, further close and community-based collaboration between the hospitals and the neighboring dental clinics are necessary. We should encourage the close communication and information-sharing of their needs concerning oral health care between the hospitals and the neighboring dental clinics in each medical community.

Probing Force for Accurate Detection of Calculus on Root Surfaces

Emi Ito, Izumi Yuki, Ryoko Funahashi, Tomomi Ohba, Mai Takahashi, Ryoichi Hosokawa, and Takeyoshi Koseki

Abstract. Probing to detect calculus on root surfaces in periodontal pockets is the most common and gold-standard technique for the diagnosis and evaluation of treatments of periodontal diseases. However, root surfaces with calculus are always atypical and it is very difficult to identify the exact location of invisible calculus within periodontal pockets. Periodontal probing also encompasses inherent measurement errors from a wide range of clinical source. The average probing forces pushing laterally and axially against the tooth model were significantly gentle in the trained examinees, who identified the two test shape, compared with unpracticed group who identified only one shape. For the accurate detection of the morphology of root surfaces, the optimal lateral and axial probing forces on the probe tip are suggested to be around 20 gramF and examinee might need to take time to concentrate sophisticated and delicate sensing. We need to develop the suitable methods to teach these sophisticated skills in early dental practical educations.

Key words. Calculus, Periodontal pocket, Periodontal probe, Probing force, Root surface

E. Ito

Division of Preventive Dentistry, Department of Oral Health and Development Sciences, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

and

Yamagata Dental Hygienist School, 2-4-35, Tokamachi, Yamagata 990-0031, Japan

I. Yuki, R. Funahashi, T. Ohba, and M. Takahashi

Yamagata Dental Hygienist School, 2-4-35, Tokamachi, Yamagata 990-0031, Japan

R. Hosokawa and T. Koseki (✉)

Division of Preventive Dentistry, Department of Oral Health and Development Sciences, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

e-mail: yobou@dent.tohoku.ac.jp

1 Introduction

Probing to detect calculus on root surfaces of periodontal pockets is the most common and gold-standard technique for the diagnosis and evaluation of treatments of periodontal diseases. This quick and simple examination method is requested to proof the accuracy and reproductively of the repeated measurements, because the probing depth and the attachment level of the periodontal pockets represent the severity and longitudinal progression of the periodontal diseases. However, root surfaces with calculus are always atypical and it is very difficult to identify the exact morphology of invisible calculus within periodontal pockets. Periodontal probing also encompasses inherent measurement errors from a wide range of clinical source, such as instrument, patients with disease status, and examiner. In this paper, we discuss the practical skills of periodontal probing for teaching accurate detection of the root surfaces.

2 Examiner's Skills of Probing on Root Surfaces

To classify the skilled and unpracticed examinees for this study, we developed the model tooth with periodontal pocket to measure the probing force during exploring root surfaces. This model tooth had the various patterns of small steps which height was 0.3 mm, with 7 mm in width on root surface. This model tooth were probed by the dental hygienists, and they answered the invisible shape of steps within the root surfaces. There are two groups of the examinees, who identified the shape of test plates, both two single steps or one double step at least one test plates (positive group), and who identified one double steps but not one double step (negative group). The average probing forces pushing laterally and axially against the test plates were significantly gentle in positive group, compared with negative group. For the accurate detection of the morphology of root surfaces, the optimal lateral and axial probing forces on the probe tip were suggested to be around 20 gramF and examinee might need to take time to concentrate sophisticated and delicate sensing.

3 Teaching Periodontal Probing Technique

Probing procedure is consisted with several elements of motion, including positioning for probing, gripping probe, inserting direction of probe tip, sliding among surface, pushing force, surface tactile sensing, read scale markings, and relocate probe "walking" to the next examination area.

It is difficult to introduce and to teach the periodontal probing skills to the students of dentist and dental hygienist by means of classroom lectures. Especially, educating the probing force is difficult to recognize and to evaluate because of its invisible dexterity.

For this purpose, students practice by themselves to probe underneath their fingernails with conventional probe to realize the force with ischemia without any pain, or to probe pushing against weight scale to read and sense the exact probing force. When using weight scale, however, practitioner concentrated to adjust the scale with pushing and could not concentrate the sense of suitable pushing force with light-fingered. Thus, we need to develop educational tools for easy learning of the probing procedure for very first training of periodontal probing for dentist and dental hygienist students.

4 Conclusions

To detect calculus in periodontal pockets, especially irregular form like steps, the optimal lateral and axial probing force was suggested to be around 20 gramF and examinee might need to take time to concentrate sophisticated and delicate sensing. We need to develop the methods to teach these sophisticated skills in early dental practical educations.

Measurement of Formaldehyde Vapor Concentration of the Surrounding Air of a Dental Clinic

Shindoh Taku, Ikawa Kyoko, Ikawa Motohide,
and Shimauchi Hidetoshi

Abstract. The formaldehyde vapor concentration of the surrounding air of a dental clinic was studied. The measurements were made at Division of Endodontics, Tohoku University Hospital. Total of 2 L of surrounding air was collected in 10 min using an automatic gas sampling device. The collection was carried out according to the sampling method of Working Environment Measurement Law. The concentration of formaldehyde vapor in the collected air was later estimated by high-performance liquid chromatography. The concentration of formaldehyde vapor of the surrounding air of the dental clinic was lower than the administrative level (0.1 ppm) of Industrial Safety and Health Law and was at an acceptable level.

Key words. Formaldehyde, Working environment, Root canal

1 Introduction

According to the modification of the Industrial Safety and Health Law and Ordinance on Prevention of Hazards due to Specified Chemical Substances [1], the administrative level of the formaldehyde vapor concentration in working space was changed

S. Taku (✉)

Environment Conservation Research Institute, Tohoku University,
6-6-04 Aramaki-aoba, Aoba-ku, Sendai 980-8579, Japan
e-mail: shindoh@env.tohoku.ac.jp

I. Kyoko

Division of Preventive Dentistry, Department of Oral Health
and Development Sciences, Tohoku University Graduate School
of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

I. Motohide and S. Hidetoshi

Division of Periodontology and Endodontology, Department
of Oral Biology, Tohoku University Graduate School of Dentistry,
4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

from 0.25 ppm to 0.1 ppm on administrative action to the evaluation of the risk of occupational diseases.

In endodontic treatment, formaldehyde is commonly used for the intracanal dressing as a compound. The authors measured the formaldehyde vapor concentration in the nearby air of the simulated intracanal dressing of Formalin Guaiacol (FG) [2]. The results indicated that the root canal dressing of FG produced the formaldehyde vapor at the acceptable concentration designated by the law. There was a difference between the vapor concentration of the air collected in the summer and that in the winter. The use of dental vacuum was advocated during the root canal dressing of FG in order to decrease the vapor concentration [2].

2 Methods

The measurement of the formaldehyde vapor concentration of the surrounding air of a dental clinic was made at one of dental chairs at Tohoku University Hospital, Division of Endodontics. There was no exhaust fan or air-conditioning can be operated locally in the clinic. Air-conditioning system was centrally managed. The nearby air to the chair was collected in total 2 L in 10 min using an automatic gas sampling device (sampling pump and DNPH gas tube) at each measurement. The collection was carried out at every 2 h during the day (9 am–5 pm); four or five times a day for consecutive 4 days. Formaldehyde vapor in the surrounding air was trapped into DNPH gas tube and afterwards desorbed with acetonitrile. The concentration of formaldehyde was later estimated by high-performance liquid chromatography.

3 Results and Discussion

There was no obvious change in the concentration between each sample throughout the experimental period. In any case, the concentration of formaldehyde vapor in the collected sample air was lower than the legally designated value (0.1 ppm). The concentration during the dressing was at most approximate 10% of the value designated by the law.

We confirmed that the vapor concentration of the air collected in Division of Endodontics, Tohoku University Hospital was at an acceptable level designated by the Industrial Safety and Health Law and the clinic was satisfactory as working environment with this view point. However, attention should be paid to the vaporization of formaldehyde into the surrounding air during the use of formaldehyde compound at endodontic treatment.

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Model of Oral Health Care Programs to Achieve Self-Actualization in Nursing Homes

Miyuki Sugiyama, Jun Aida, and Takeyoshi Koseki

Abstract. In aging society, the life of elderly people should be brighten and be enriched by supporting and maintaining a high standard of their quality of life (QOL). Especially in nursing home, the level of QOL of the residents should be maintained regardless their disability or the degree of care necessary. From our analysis of questionnaire and assessment of the facility-users and care-providers, we proposed an oral health care model referring Maslow's hierarchy of needs. This model represents the role of oral health care to achieve self-esteem and self-actualization of both facility-users and care-providers. Promoting oral health care in nursing home might create hospitable and humanized workplace and living place. Furthermore, the proposed oral health care model would construct more integrated relation between facility-users and care-givers, and it would provide well-being to both, with high standard of QOL.

Key words. Care-provider, Facility-user, Needs, Nursing home, Quality of life

1 Introduction

In the hyper-aged society-Japan, the life of elderly people should be brighten and be enriched by supporting the high standard of their quality of life (QOL). Especially in nursing home, the high standard of QOL of the facility-users should be maintained regardless their disability or the degree of care necessary, however, it might make

M. Sugiyama and T. Koseki (✉)

Division of Preventive Dentistry, Tohoku University Graduate School
of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai, Miyagi 980-8575, Japan
e-mail: yobou@dent.tohoku.ac.jp

J. Aida

Division of International Oral Health, Tohoku University Graduate School
of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai, Miyagi 980-8575, Japan

care-provider increasing burden to push up the higher level of QOL of whole facility-users. In this paper, we discuss the measure to connect the worthwhile work of care-providers and high level of QOL of facility users, and propose a model of needs in oral health care to achieve self-actualization of both facility-users and care-providers.

2 Quality of Life in Nursing Home

The facility-users and care-providers in the same healthcare facility for the elderly, filled each questionnaire, and also assessed the condition of health and disability of the facility-users. The researches in the facility residential care revealed that the decreased level of serum albumin was correlated to the degree of care necessary in multiple linear regression analysis. This indicated the importance of maintaining the nutritional condition with intensive support of eating. On the other hands, care-providers motivated and satisfied their care work by the user’s recuperation, thankful acknowledgment, and recognition of supporting the QOL of the facility-users.

3 Model of Needs for Facility-Users and Care-Providers

In 1943, Abraham Maslow proposed a theory in human developmental psychology, so-called Maslow’s hierarchy of needs [1]. From our analysis of questionnaire and assessment of the facility-users and care-providers, we proposed the hierarchy of

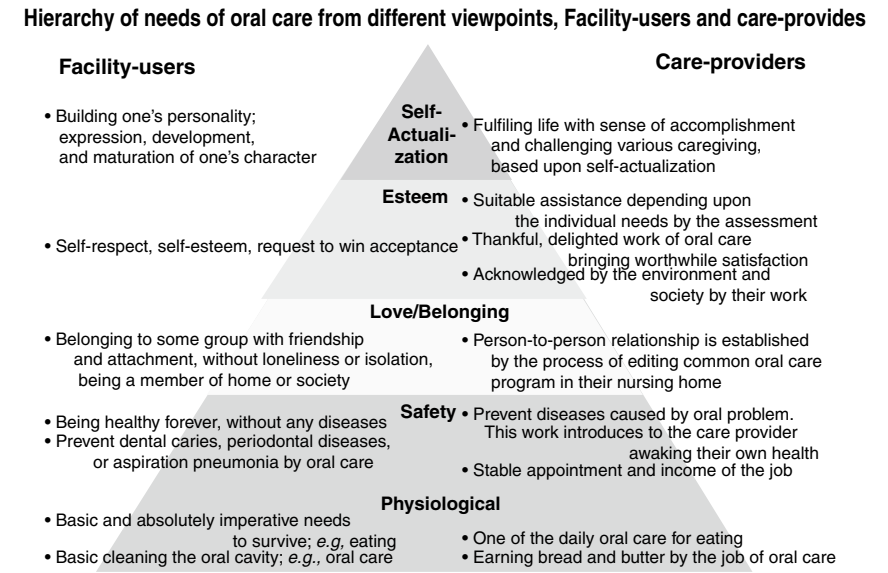


Fig. 1. Hierarchy of needs of oral care from different viewpoints, facility-users and care-providers

needs of oral health care to maintain high standard of both QOL, by performing oral health care to facility-users by care-providers. This model represents the role of oral health care to achieve self-esteem and self-actualization of both facility-users and care-providers. Promoting oral health care in nursing home might be create hospitable and humanized workplace and living place for both (Fig. 1).

4 Conclusion

In nursing home, it is important to preserve QOL of both facility-users and care-providers. The proposed oral health care model would construct more integrated relation between facility-users and care-providers, and it would provide well-being to both.

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Promotion of Professional Oral Care for Hospitalized and Home-Care Patients

Toru Tamahara, Jun Harako, Ryoichi Hosokawa, Kazunori Kimura, Moritoshi Komagata, Koji Hanaoka, Seiichi Aonuma, Fusako Okuya, Kaoru Itagaki, Yukiko Akai, Kazuhiko Tamura, Hidetoshi Sonobe, and Takeyoshi Koseki

Abstract. To provide the intensive oral care to the hospitalized and home-care patients, we discuss the current status and improving measures of promoting oral care in Sendai city. The questionnaire survey among the dentists who are working at the dental offices, revealed that one-fourth of the dentists has experiences of visiting to the patient home, other medical facilities, and the nursing homes for the elderly. Half of the dentists who had no experiences to visit patients out of their dental offices, considered that they had prepared ready to go out from their offices by the requests for oral care on demand. To promote the professional oral care for the hospitalized and home-care patients, it would be helpful to open the liaison offices to share information and to collaborate between the medical and dental facilities each other about the needs of oral care.

T. Tamahara, J. Harako, and R. Hosokawa
Division of Preventive Dentistry, Department of Oral Health
and Development Sciences, Tohoku University Graduate School
of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

K. Kimura, M. Komagata, K. Hanaoka, S. Aonuma, F. Okuya, K. Itagaki, Y. Akai,
K. Tamura, and H. Sonobe
Division of Oral and Dental Health Care, Council for Community Healthcare of Sendai,
Sendai 980-8575, Japan

T. Koseki (✉)
Division of Preventive Dentistry, Department of Oral Health
and Development Sciences, Tohoku University Graduate School
of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
and
Division of Oral and Dental Health Care, Council for Community
Healthcare of Sendai, Sendai 980-8575, Japan
and
Community Oral Health Promotion Office, Tohoku University Graduate School
of Dentistry, Sendai 980-8575, Japan
e-mail: yobou@dent.tohoku.ac.jp

Key words. Community dentistry, Hospitalized patients, Liaison office, Oral care

1 Introduction

Almost all patients who require oral care show some decreased activity of daily living (ADL), such as being bedridden, dementia, and suffering with consciousness disorder or severely impaired oral function. Disability of oral function further induces several medical risks and problems, especially in hospitalized and home-care patients. Under these circumstances, oral care becomes one of the daily routine nursing care in hospitals. The nurses and caregivers who provide oral care to the patients, are asking to the dental profession for the help of supervision in many occasions. To provide the intensive oral care to the hospitalized and home-care patients, we discussed the current status and improving measures of oral care from a standpoint of dentists in Sendai city.

2 Current Status of Professional Oral Care Out of the Dental Offices

One-fourth of the dentists reported the experiences of visiting to patient homes, other medical facilities, such as the health care facilities and the nursing homes for the elderly. Among them, many dentists already had some information-sharing with the patient homes or other medical facilities to provide oral care, and most of them wanted to build closer relationship with them. Half of the dentists who had no experiences to visit patients out of their dental offices, considered that they had prepared ready to go out from their offices by the requests for oral care on demand. However, few of these dentists desire to shift their work to professional oral care of out-of-office patients.

3 The Issues for Professional Oral Care Out of Dental Offices

The major reasons why dentists do not want to shift their work to care of the out-of-office patients are just disinterestedness or financial discord, because medical service fee and cost for care do not break even. Furthermore, one of the underlying cause why they did not go outside the office might be lack of a chance to build a relationship with any medical institution or healthcare facility, even if the dentists wished to establish the partnerships for oral care. The dentists who had experiences of visiting for oral care out of dental offices, complained several problems, including the low level of health care services fee provided by

health insurance, the difficulty to get understanding and cooperation with the faculty staff for oral care because of busyness, and lack of the knowledge of staffs for oral health and oral care. On the other hand, the dentists who had no experiences of visiting out of dental offices, complained about lack of the knowledge and consciousness of the dentists for oral care, lack of the time to manage oral care out of the dental offices because of busyness, and the difficulty of sharing information with other medical faculties.

4 To Promote Oral Care for Hospitalized and Home-Care Patients

Regardless of the experiences of professional oral care out of the dental office, dentists should accelerate and keep up the efforts of the professional oral care with the partnership with the medical facilities. To promote the professional oral care for the hospitalized and home-care patients, it would be helpful to open the liaison offices to share information and to collaborate between the medical and dental facilities each other about the needs of oral care.

Relationship of Periodontal Disease and Tooth Loss to Glucose Metabolism Disorder: The Ohasama Study

Takashi Ohi, Yoshitada Miyoshi, Takahisa Murakami,
Shiho Itabashi, Yoshinori Hattori, Akito Tsuboi, Yutaka Imai,
and Makoto Watanabe

Abstract. The purpose of this study was to examine the association of periodontal disease and tooth loss with glucose metabolism disorder. Cross-sectional data on 507 individuals aged ≥ 55 years old were obtained from the general population of Ohasama, a rural community in Japan. Periodontal status was examined using probing depth (PD) and clinical attachment loss (CAL). Subjects with at least 10 teeth were divided into two groups based on mean PD and CAL, with cut-off of 2.0 mm and 2.5 mm, respectively. The other subjects with 0–9 teeth were defined as multiple tooth loss group. Glucose metabolism disorder was defined as serum glycosylated hemoglobin (HbA1c) level of at least 5.8% and/or the use of medication for diabetes. Multivariate logistic regression analysis indicated that $CAL \geq 2.0$ mm and multiple tooth loss were independently associated with the presence of glucose metabolism disorder. Our results suggest that a history of periodontal disease assessed by CAL and multiple tooth loss may be associated with an increased risk of glucose metabolism disorder.

Key words. Clinical attachment loss, Glucose metabolism disorder, Periodontal disease, Tooth loss

T. Ohi (✉), Y. Miyoshi, T. Murakami, S. Itabashi, Y. Hattori, and A. Tsuboi
Division of Aging and Geriatric Dentistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: tohi@dent.tohoku.ac.jp

Y. Imai
Department of Planning for Drug Development and Clinical Evaluation,
Tohoku University Graduate School of Pharmaceutical Sciences,
2-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

M. Watanabe
Tohoku Fukushi University, 6-149-1 Kunimigaoka, Aoba-ku, Sendai 989-3201, Japan

1 Introduction

Recent studies have suggested that periodontal disease is related to metabolic risk factors [1–3]. Glucose metabolism is one of these factors. The purpose of this study was to examine the association of periodontal disease and tooth loss with glucose metabolism disorder in community-dwelling Japanese population.

2 Methods

Cross-sectional data on 507 individuals (female, 65.9%) aged ≥ 55 years old (mean 68.5 $\pm$ 6.9) were obtained from the general population of Ohasama, a rural community in northern Japan. Periodontal status was examined using the probing depth (PD) and clinical attachment loss (CAL). Subjects with at least 10 teeth were divided into two groups based on mean PD and CAL, with cut-off of 2.0 mm and 2.5 mm, respectively. The other subjects with 0–9 teeth were defined as multiple tooth loss group. Glucose metabolism disorder was defined as serum glycosylated hemoglobin (HbA1c) level of at least 5.8% and/or the use of medication for diabetes. The association of periodontal disease and tooth loss with glucose metabolism disorder was explored by multivariate logistic regression analysis adjusted for age, gender, body mass index, smoking, alcohol consumption; and a history of hypertension and hypercholesterolemia.

3 Results

Association between glucose metabolism disorder and severity of periodontal disease assessed using PD was not significant. Odds ratios of $CAL \geq 2.0$ mm (2.68, 95% confidence interval 1.09–6.58) and multiple tooth loss (2.90, 95% confidence interval 1.13–7.46) for the presence of glucose metabolism disorder were significantly higher than that of $CAL < 2.0$ mm.

4 Conclusion

Our results suggest that a history of periodontal disease assessed by CAL and multiple tooth loss may be associated with an increased risk of glucose metabolism disorder.

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Relationships Between Oral Health-Related Quality of Life and the Patterns of Remaining Teeth in the Middle-Aged and Elderly

Yoshitada Miyoshi, Takashi Ohi, Takahisa Murakami,
Shiho Itabashi, Yoshinori Hattori, Akito Tsuboi, Yutaka Imai,
and Makoto Watanabe

Abstract. The aim of this study was to investigate the relationship between oral health-related quality of life (OHRQoL) and the patterns of remaining teeth in different age groups. A total of 512 independent community-dwelling people (age: 68.0 ± 7.0 , female: 65.9%) in Ohasama participated in this study. Subjects were divided into four categories by the patterns of remaining teeth and divided into three age groups (≤ 65 years, 66–71 years, ≥ 72 years). To evaluate OHRQoL, the Oral Impacts on Daily Performances (OIDP) scale was administered. According to the result of multiple regression analyses, in all age groups, the patterns of remaining teeth had significant associations with OHRQoL. Correlations appeared to be stronger between the patterns of remaining teeth and OHRQoL in the elderly group than in the middle-aged group. These results suggest that the maintenance of occlusal support and tooth retention are important to maintain quality of life levels later in life.

Key words. Oral health-related quality of life, The pattern of remaining teeth

Y. Miyoshi (✉), T. Ohi, T. Murakami, S. Itabashi, Y. Hattori, and A. Tsuboi
Division of Aging and Geriatric Dentistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: miyoshi@mandible.dent.tohoku.ac.jp

Y. Imai
Department of Clinical Pharmacology and Therapeutics, Tohoku University
Graduate School of Pharmaceutical Sciences and Medicine, 1-1 Seiryomachi,
Aoba-ku, Sendai 980-8574, Japan

M. Watanabe
Tohoku Fukushi University, 149-1 Kunimigaoka-6, Aoba-ku, Sendai 980-8574, Japan

1 Introduction

It is well known that it is important to have a lot of teeth in the oral cavity in order to maintain QoL in the elderly. However, it is thought that not only the number of teeth but also occlusal support is an important element so that a tooth functions well. The aim of this study was to investigate the relationship between oral health-related quality of life (OHRQoL) and the patterns of remaining teeth in different age groups.

2 Study Design

A total of 512 independent community-dwelling people older than 55 years (age: 68.0 ± 7.0 , female: 65.9%) in Ohasama, a Japanese rural area, participated in this study. Subjects were divided into four categories by the patterns of remaining teeth according to the Miyachi classification (area A–D; A: occlusal supports ≥ 10 , B: occlusal supports 9–5, C: occlusal supports ≤ 4 and remaining teeth 10–0, D: occlusal supports ≤ 4 and remaining teeth 11–18) [1], and divided into three age groups (≤ 65 years, 66–71 years, ≥ 72 years). To evaluate OHRQoL, the Oral Impacts on Daily Performances (OIDP) scale [2] was administered. Multiple regression analyses were applied for three age groups to investigate correlations between the four Miyachi categories and OHRQoL, adjusted for age, gender, chronic disease, smoking and drinking habits, depressive tendency, cognitive function and utilization of dental services over the past 1 year.

3 Conclusion

In all age groups, the patterns of remaining teeth had significant associations with OHRQoL and Odds ratio of area D to area A was the highest of all the four categories. Subjects with many teeth and few occlusal supports (area D) had the highest risk of low OHRQoL. Correlations of the ≥ 72 years age group were stronger than those of the ≤ 65 years age group or 66–71 years age group.

Correlations appeared to be stronger between the patterns of remaining teeth and OHRQoL in the elderly group than in the middle-aged group. It suggests that both the maintenance of occlusal support and tooth retention are important to maintain quality of life levels later in life.

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Community Oral Health Promotion Program Fostering Self-Management for the Elderly

Tomoko Nishihira, Miho Nishitani, Takuichi Sato, Yuki Abiko,
Kenji Matsushita, Misao Hamada, Motomi Tao, and Reiko Sakashita

Abstract. This study aimed to evaluate a health promotion program for the elderly to foster self-management of oral health. The results of the first district evaluated among four districts are reviewed. Subjects consisted of 31 people (average age, 73.1 ± 7.4 years). The intervention consisted of a group study and private consultations for 3 months. As a consequence, subjects cleaned their teeth more often than before ($P < 0.05$). Deposits of plaque and tartar were significantly lower after intervention ($P < 0.01$ – 0.001), while scores for oral function did not significantly improve. Scores for QOL and cognitive function improved significantly ($P < 0.05$). These results suggest that this program promotes not only oral self-care, resulting in good oral health conditions, but also improvement of cognitive function of the elderly.

Key words. Cognitive function, Elderly, Health promotion, Self-management

T. Nishihira, M. Nishitani, M. Tao, and R. Sakashita (✉)
Nursing Foundation, College of Nursing Art and Science,
University of Hyogo, Akashi 673-8588, Japan
e-mail: sakashita@cnas.u-hyogo.ac.jp

T. Sato
Division of Oral Ecology and Biochemistry, Tohoku University Graduate
School of Dentistry, Sendai 980-8575, Japan

Y. Abiko
Division of Oral Ecology and Biochemistry, Tohoku University Graduate
School of Dentistry, Sendai 980-8575, Japan
and
Department of Microbiology, School of Dentistry,
Aichi-Gakuin University, Nagoya 464-8650, Japan

K. Matsushita
National Center for Geriatrics and Gerontology, Obu 474-8511, Japan

M. Hamada
Social Welfare Corporation Lavita, Osaka 554-0002, Japan

1 Introduction

To promote oral health for elderly people, it is important to foster their self management to practice proper health behaviors such as oral cleaning and visiting a dentist. Based on the previous studies [1, 2], a health promotion program for the elderly to foster self-management of oral health was developed. This study aimed to review the results of the first district evaluated among four districts.

2 Outline of Community Oral Health Promotion Program

Informed consent was obtained from 31 elderly subjects (average age, 73.1 ± 7.4 ; range, 61–94 years). The intervention program consisted of the group studies and private consultations for 3 months. The group study included (1) monitoring the oral condition and practicing oral self-care, (2) monitoring the oral function and practicing oral exercise, and (3) group discussion on continuing oral self-care. Outcomes were evaluated at the beginning, the end, and 3 months after the investigation by the scores in (1) oral self-care (2) oral condition (decayed teeth, CPI, deposits of plaque and tartar), (3) oral function (RSST, Oral Diadochokinesis), (4) QOL (SF-8 v2™, GOHAI), and (5) cognitive function (MMSE-J).

3 Effectiveness of the Program

On the oral self-care, subjects cleaned their teeth more often than before. The use of dental floss was significantly increased in number, and 20 participants (65%) visited dentist during the program. Deposits of plaque and tartar were significantly reduced after intervention ($P < 0.01$ – 0.001), while, the scores of oral function did not significantly improve. The scores of QOL and cognitive function significantly improved ($P < 0.05$). These results suggest that this program promotes not only oral self-care, resulting in good oral health conditions, but also improvement of cognitive function and QOL of the elderly.

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**Harvard–Forsyth–Tohoku Research
Workshop Tohoku University
Graduate School of Dentistry**

Highly Biodegradable Bone Substitute Materials with OCP

Osamu Suzuki and Takahisa Anada

Abstract. Biodegradation of the implanted bone substitute materials is an essential factor to determine the amount of newly formed bone. It is considered that one of ideal properties in the biomaterials is to have a biodegradation rate that coincides with the rate of new bone formation by osteoblasts. The purpose of the present article is to review on the biodegradation on one of calcium phosphate ceramics, octacalcium phosphate (OCP), and OCP-based polymer composites, such as OCP/alginate, we developed. OCP is more soluble than typical biodegradable β -tricalcium phosphate (β -TCP) ceramic but less soluble than dicalcium phosphate dihydrate (DCPD), a raw material used to form apatite cements. It has been shown that if OCP crystals are homogenously dispersed in the polymer matrices, then the composites enhance bone formation coupled with osteoclastic resorption of OCP crystals dispersed. The mechanism of the OCP biodegradation will be discussed.

Key words. Biodegradation, Bone formation, Octacalcium phosphate, Osteoblasts, Osteoclasts

1 Introduction

Much attention has been paid to the development of biodegradable bone substitute materials because they are advantageous to conducting large amount of new bone matrix [1] in bone defects. Biodegradable materials include inorganic calcium phosphate ceramics, such as β -tricalcium phosphate (β -TCP, β -Ca₃(PO₄)₂) and carbonate-containing hydroxyapatite (HA) [2], natural polymers and synthetic polymers, such as polylactic acid [3]. The mechanisms of the biodegradation of these materials, especially those of calcium phosphate ceramics, are still unclear. In general, the material

O. Suzuki (✉) and T. Anada

Division of Craniofacial Function Engineering, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: suzuki-o@m.tohoku.ac.jp

own degradation should be the most influential factor but the cellular resorption may also be involved in the degradation process. In this article, the property of octacalcium phosphate (OCP, $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$), which has currently been recognized as a cell-stimulating material with biodegradable characteristics [4], is summarized from the view point of the material chemistry and the cellular response.

2 Biodegradable Calcium Phosphate Materials

Various calcium phosphate compounds in relation to bone substitute materials, including OCP, are shown in Table 1. Acidic calcium phosphates, including OCP, DCPD ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) and anhydrous form of DCP (DCPA, CaHPO_4), have higher solubilities than HA ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) at neutral pH [5]. DCPA and DCPD are source materials to form HA cement [6]. Amorphous calcium phosphate (ACP), recently recognized as amorphous tricalcium phosphate (ATPC, $\text{Ca}_3(\text{PO}_4)_2 \cdot n\text{H}_2\text{O}$) [7], are also one of highly soluble salts and more soluble than HA at that condition [8, 9]. DCPA and ACP as well as OCP have been shown to exhibit more osteoconductive than HA [8]. OCP and ACP are recognized to be biodegradable materials [4, 7, 8, 10]. Sintered dense HA ceramic is recognized as non-resorbable biomaterial [2]. However, HA becomes resorbable if carbonate ion is incorporated [11] or the crystal size decreases into nano-scale [12]. Composites consisted of the biodegradable calcium phosphates and natural polymers have been developed as bone substitute materials [12] and scaffold materials for mesenchymal stem cells (MSCs) that are also used as bone substitutes [13]. The effect of OCP inclusion within the collagen (Col) [14], gelatin [15] and alginate (Alg) [16] on bone regeneration has been confirmed. These composites can also be used as seeding bone marrow derived cells to enhance the repair in bone defects [16, 17].

Table 1. Various calcium phosphate compounds in relation to bone substitute materials

Calcium phosphate	Abbreviation	Chemical composition	Notes (References)
Dicalcium phosphate, anhydrate	DCPA	CaHPO_4	Raw material in apatite cement [6]; osteoconductive [9]
Dicalcium phosphate, dihydrate	DCPD	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	Raw material in apatite cement [6]
Octacalcium phosphate	OCP	$\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$	Osteoconductive and resorbable [4, 8, 14, 20, 21]
Amorphous (tri-) calcium phosphate	ACP (ATCP)	$\text{Ca}_3(\text{PO}_4)_2 \cdot n\text{H}_2\text{O}$	Osteoconductive and resorbable [7, 8]
β -tricalcium phosphate	β -TCP	$\beta\text{-Ca}_3(\text{PO}_4)_2$	Osteoconductive and resorbable [2, 22]
Hydroxyapatite	HA	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	Non-resorbable [2]; Resorbable [11, 12]

3 Capability of the Materials to Simulate Cellular Function

It is well accepted that calcium phosphate ceramics, especially HA ceramic, are better scaffolds for bone marrow derived cells [13] due to their highly biocompatible characteristics of the materials. However, it is still unclear whether calcium phosphate materials work as stimulant for osteoblasts and osteoclasts. Recent studies demonstrated that OCP is capable of enhancing osteoblastic cell differentiation more than HA [4, 18] with significant increase of the marker genes, such as osteirx, alkaline phosphatase (ALP) and Col 1 in vitro [18]. On the other hand, OCP is also able to induce osteoclast formation from co-culturing in bone marrow-derived cells and osteoblasts with increasing receptor activator of NF-kappaB ligand (RANKL) in osteoblasts even in the absence of vitamin D₃, a factor to raise RAKL expression in osteoblasts [19]. OCP can convert to HA in vitro culture condition and in vivo implantation site [4, 8, 18]. It has been confirmed that the osteoblastic cell differentiation and the increase of RANKL expression in osteoblasts can be stimulated through a crystal-cellular interaction enhanced by a physicochemical OCP-HA conversion process [4]. Direct osteoclastic resorption of OCP implant has been observed in various bone defects experimentally created. Figure 1 shows OCP granules implanted in rabbit femur bone marrow space (Fig. 1a) [20] and mouse calvaria (Fig. 1b) [21]. They show a character of multinucleated giant cells (Fig. 1a) and are stained by cathepsin-K (Fig. 1b), a marker for osteoclasts which are recognized to be the cells actively resorbing the bone matrix. Other in vivo studies reported that β -TCP [22] or nano-HA in Col matrix [12] can also be resorbed by osteoclastic cells. The cell-stimulating capability of OCP, especially in the stimulation to form osteoclasts, has been confirmed in vitro without any additional factors, such as growth factors or matrix proteins.

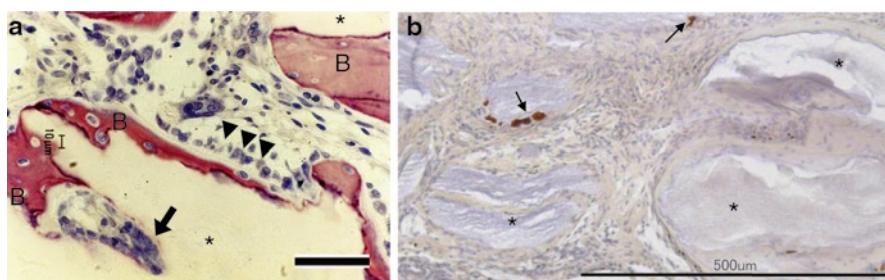


Fig. 1. Photographs of histological sections of OCP granules of 500–1000 μm in diameter stained by hematoxylin and eosin using un-decalcified section of rabbit femur bone defect at 8 weeks after the implantation (a) and by cathepsin-K antibody using decalcified section of mouse calvaria critical-sized defect at 10 weeks after the implantation (b). Osteoblasts with a cuboidal shape aligned on the OCP granule surface (arrow heads) in (a); new bone matrix (B); osteoclast-like multinuclear cells resorbing the OCP granule surface (arrow) in (a); cathepsin-k positive osteoclast-like cells (arrows) in (b). Asterisks, OCP granules. Bars, 50 μm (a) and 500 μm (b). Reproduced from Suzuki [20] and Murakami et al. [21] with permissions from Elsevier Ltd.

4 Bone Regeneration Coupled with Biodegradation

The composites of HA/Col [12], OCP/Col [14] and OCP/Alg [16] have been reported to be biodegradable materials and tend to be replaced with new bone with implantation period. Col molecule is recognized to have cell-adhesive motif in the structure while alginate does not have such a motif in the molecule [16]. Nevertheless, OCP/Alg exhibits better osteoblastic cellular attachment capacity in vitro. OCP/Alg can regenerate new bone in mouse calvaria critical-sized bone defect [16]. This is due to the effects of: (1) homogenous OCP crystal distribution throughout the Alg matrix; (2) the structure having pores larger than the cells up to 52 μm are secured in the matrix skeleton [16]. In fact, mouse bone marrow stromal cells can well proliferate if increasing the pore size from 20 to 52 μm in OCP/Alg composite in vitro. When the new bone is formed with OCP/Alg implantation in the mouse calvaria critical-sized bone defect, tartrate-resistant acid phosphatase (TRAP)-positive osteoclastic cells appear in a close association with OCP crystals in Alg matrix [16]. The biological response observed in vitro and in vivo could be brought about by the stimulatory capacity of OCP crystals dispersed in Alg matrix. OCP crystals may be a candidate material used to provide the bone regenerative property which is coupled with biodegradable characteristics. Possible mechanism induced by bone substitute materials including OCP crystals, such as OCP/Alg, stemmed from the physicochemical property of OCP, is drawn in Fig. 2.

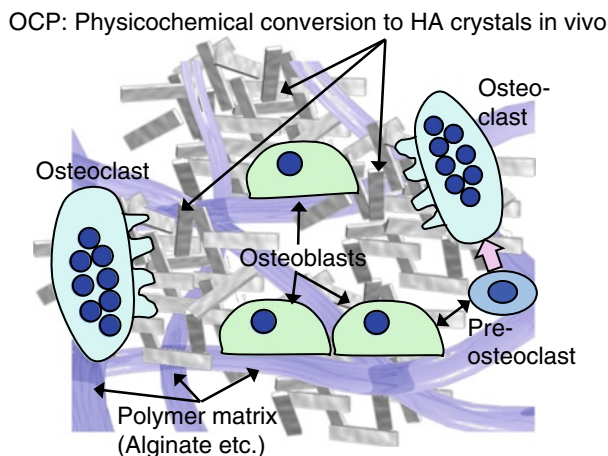


Fig. 2. Schematic view of new bone formation by osteoblasts coupled with biodegradation by osteoclastic cells in OCP/polymer matrix, such as OCP/alginate, stimulated by OCP crystals dispersed in the polymer matrix. Recent studies have shown that the osteoblastic cell differentiation and the increase of RANKL expression in osteoblasts can be stimulated through a crystal-cellular interaction enhanced by a physicochemical OCP-HA conversion process [4, 18, 19]

5 Conclusion

The biodegradation of the implanted biomaterials with a suitable rate could be followed by better new bone formation that may bring about organization of bone with better quality. The biodegradation rate can be controlled by the material properties, such as the solubility and the cellular responsiveness, which could be determined depending on the type of calcium phosphate. The studies of OCP implantation in bone defects suggest that OCP may be one of materials that meet the criteria which could be involved in physiological remodeling process in bone. Further study is underway to establish a linkage between the materials chemistry of calcium phosphates, especially OCP, and the biological responses.

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Epithelial Cell Lines in the Field of Dental research: Review

Satoshi Fukumoto, Makiko Arakaki, Tsutomu Iwamoto, Aya Yamada, Ryoko Miyamoto, Masahiro Naruse, and Takashi Nakamura

Abstract. The interaction between the epithelium and mesenchyme induces specific molecular and cellular changes that lead to organogenesis. These interactions are particularly crucial during the initiation of the development of ectodermal organs, such as teeth, skin, hair, and mammary and prostate glands. The oral epithelium provides the initial signaling for neuronal crest-derived ectomesenchyme development, and then both tissues interact during tooth formation. Various transcription factors, growth factors, and extracellular matrices are expressed by enamel matrix-producing ameloblasts during tooth development.

Dental epithelium was lost after tooth eruption in human. To analysis of dental cell proliferation and differentiation, we should use the dental epithelial cells from tooth germ, for example third molar, supernumerary tooth or continuous erupting rodent incisor. However, primary culture of dental epithelium has a limited number of cells and passage times. Because of these reasons, cell lines from dental tissue are useful to clear the molecular mechanism of these processes. Here we introduce cell lines from dental tissues, especially dental epithelium.

Key words. Ameloblastin, Ameloblastoma, Dental epithelium, Tooth development

1 Tooth Development and Dental Epithelium

Ectodermal organ development is a complex process initiated by inductive tissue interaction [1]. In tooth development, the dental epithelium and mesenchyme interact reciprocally for growth and differentiation to form the proper number and shape

S. Fukumoto (✉), M. Arakaki, T. Iwamoto, A. Yamada, R. Miyamoto, M. Naruse, and T. Nakamura
Division of Pediatric Dentistry, Department of Oral Health
and Development Sciences, Tohoku University Graduate School of Dentistry,
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: fukumoto@dent.tohoku.ac.jp

of teeth [2, 3]. Tooth development is a continuous process that can be divided into the initiation, bud, cap, and bell stages [4]. In mice, tooth development begins at embryonic day 11.5 (E11.5) by thickening of the dental epithelium. The dental lamina undergoes further proliferation and subsequently develops into the tooth bud. The tooth bud is formed by the invagination of the placode and the condensation of mesenchyme cells adjacent to the bud. At the bud stage (E13.5), dental epithelial cells proliferate diffusely. At the cap stage (E14.5), dental epithelial cells differentiate into several cell types. Cell death by apoptosis within the enamel knot is critical for cusp formation in molars. These epithelial cells proliferate at different dividing rates to form the unique shape of the tooth organ. For example, a non-proliferating epithelial cell mass forms the enamel knot at the center of the tooth bud. At the bell stage (E17.5), the dental mesenchyme differentiates into dentin matrix-secreting odontoblasts that form dentin, and then the inner dental epithelial cells differentiate into enamel matrix-secreting ameloblasts.

Dental epithelial cells in tooth buds differentiate into various cell types during development of tooth germs—inner enamel epithelium, stratum intermedium, stellate reticulum, and outer enamel epithelium [5]. The inner enamel epithelial cells subsequently differentiate into ameloblasts, which then form enamel. The mechanisms responsible for this cellular differentiation are not fully understood. Further, the formation of a tooth root occurs by the sequential and reciprocal interactions between the Hertwig's epithelial root sheath (HERS) and the surrounding mesenchyme, as well as by crown development [2]. HERS consists of the epithelial bilayer derived from the cervical loop epithelium at the cuff of the enamel organ. After the completion of crown development, HERS fuses below the level of the cervical margin of the crown [6]. Many studies have indicated that HERS is involved in the induction of odontoblast differentiation and subsequent dentin deposition during root formation through epithelial–mesenchymal interactions [7].

Production of enamel matrix by ameloblast in enamel organ epithelium is critical for the formation of enamel, mostly composed of inorganic hydroxyapatite (HA) crystals [8, 9]. Ameloblasts differentiation is controlled by sequential epithelial–mesenchymal interactions, and their origin and maturation mechanisms are still not clear. Fully differentiated ameloblasts are tall columnar cells with polarized distribution of their cytoplasmic organelles, secreting enamel matrix proteins, mainly composed of Amelogenin. Ameloblastin (AMBN), also known as amelin and sheathlin, is a tooth-specific extracellular matrix and the most abundant non-amelogenin enamel matrix protein [10]. AMBN is expressed primarily by ameloblasts, which are differentiated from the oral ectoderm and form a polarized single cell layer underlying the enamel matrix. In a previous study, we created *Ambn*-null mice and demonstrated that AMBN is required for cell attachment and polarization and for maintaining the differentiation state of ameloblasts and is essential for enamel formation [11]. Overexpression of *Ambn* in transgenic mice causes abnormal enamel crystallite formation and enamel rod morphology [12]. These results suggest that enamel formation and rod morphology are influenced by temporal and spatial expressions of AMBN.

While the developmental regulation on ameloblast or biochemical nature of enamel matrix was well analyzed in vitro and in vivo, the detailed cellular character of ameloblast is still not clear because of the lack of a method to continuously culture it in vitro. In vitro experiments are crucial to our understanding of the mechanism of dental epithelial differentiation. Primary cultured ameloblast was reported to keep differentiated character in vitro, including the expressions of ameloblast specific genes and the potential to form calcified nodules, but have restricted potential of cellular proliferation [13–15]. There are several reports on immortalization of ameloblast-lineage cells using T-antigen of SV40 or polyoma viruses [16, 17]. However, these systems did not allow the process of dental epithelial differentiation to be observed, since these cell cultures were a mixture of various dental epithelial cells and/or contained cells expressing amelogenin at the outset.

2 Dental Epithelial Cell from Tooth Germ

An in vitro cell culture system of dental epithelium is useful for the investigations of cellular differentiation and function of ameloblast in amelogenesis and of regenerative therapy in human tooth. Normal somatic cells involving the epithelial cells of enamel organ are not capable of indefinite proliferation, because their life span is limited by cellular senescence, which is controlled at least in part by telomere shortening [18]. Because of the limitation of primary culture of ameloblast-lineage cells, it is not clear the molecular mechanism of ameloblast differentiation.

Recently, several kinds of dental epithelial cell line were established (Table 1). LS-8 cell line was derived from first mandibular and maxillary molars of new born Swiss-Webster mice and established by the transfection of the plasmid, pRSV-T, SV-40 ori construct containing the SV-40 early-region genes, the Rous sarcoma virus long-terminal repeat and the polyoma T-antigen [17]. The cells could be passaged many times. They expressed a keratin-containing cytoskeleton, and approx. 60% of the cells expressed amelogenin. This cell line was used to analyze the fluoride induced endoplasmic reticulum stress [19]. While they kept expression of some ameloblast specific genes including amelogenin, it is not clear whether they keep potential to induce calcification in vitro.

Table 1. List of dental epithelial cell lines

Cell line name	Origin	Transfection	Reference
LS-8	Mouse molar	SV-40	[17, 19]
HAT-7	Cervical loop epithelium from rat incisor	None	[5, 20, 21]
SF2	Rat incisor	None	[22, 23]
ALC	Mouse molar	None	[24]
HERS01a	Mouse molar	None	[25]
AM-1	Human ameloblastoma	hPV type16	[23, 26]
HAM1, 2, 3	Human ameloblastoma	hTERT	[27]

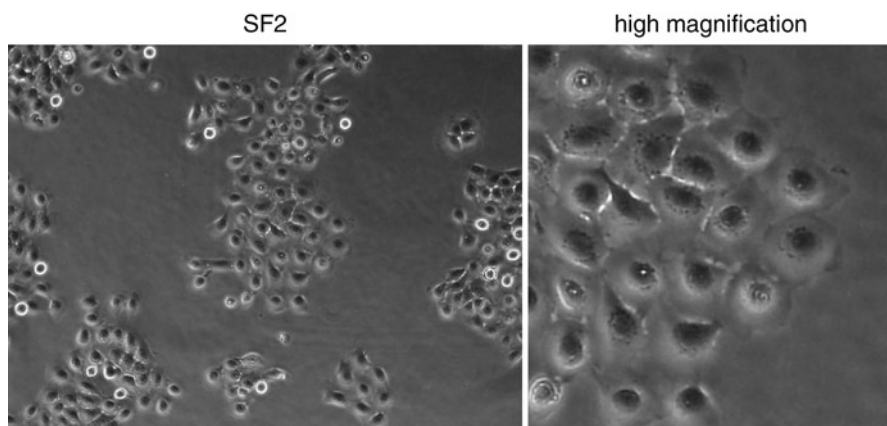


Fig. 1 Morphology of dental epithelial cell line SF2

HAT-7 is immortalized dental epithelial progenitor cell line derived from the cervical-loop epithelium of a rat lower incisor without gene transfection [5]. The expression patterns of cytokeratin 14, nerve growth factor receptor p75, amelogenin, Notch2, and alkaline phosphatase were examined by immunohistochemistry in both lower and higher cell densities. The patterns of each were compared in the dental epithelium of rat lower incisors. The results demonstrated that these cells could produce ameloblast lineage cells, stratum intermedium cells, stellate reticulum, and outer enamel epithelium. Furthermore, FGF10 stimulated proliferation of dental progenitor cells and subsequently increased the number of cells expressing alkaline phosphatase. These results suggest that fibroblast growth factor 10 plays a role in coupling mitogenesis of the cervical-loop cells and the production of stratum intermedium cells in rat incisors. HAT-7 cells were used to analyze the effect of neurotrophic factor NT-4 on the differentiation of dental epithelium [20], and the responsible for basement membrane component laminin alpha2 [21]. SF2 was established from incisor using the method similar to the establishment of HAT-7 cells [22]. SF2 cells had a typical epithelial morphology (Fig. 1). This cell line was no expression of AMBN before differentiated induction by BMP2 or NT-4. However, AMBN expression was higher than HAT-7 cells after stimulation of growth factors, including NT-4 (Fig. 2). Because of these results, SF2 is useful for analyze the AMBN expression and function, and ameloblast differentiation [22, 23].

ALC was derived from mandibular molar tooth germ of C57BL/6J without transfection [24]. The established cell line (ameloblast-lineage cell; ALC) maintained the expression of several ameloblast specific genes (Amelogenin, Tuftelin, and Enamelin) in long-term culture. They formed calcified nodules after the induction by medium switching from SMEM to DMEM, having high-level alkaline-phosphatase activity. The size and number of calcified nodule formation were enhanced by TGF- β treatment. Six weeks after sub-cutaneous implantation of ALC to athymic nude mice, we ectopically observed enamel epithelium like structure

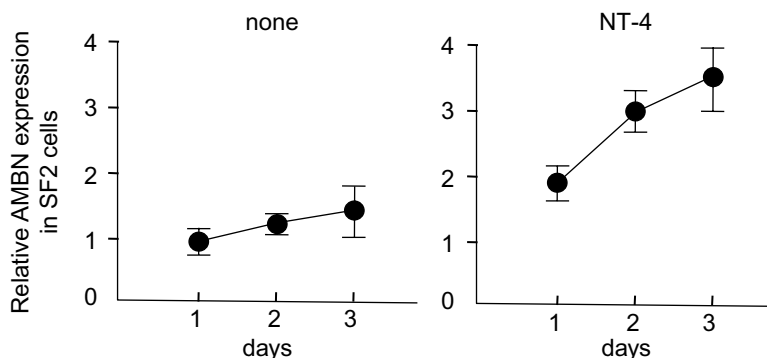


Fig. 2. Induced expression of ameloblastin (AMBN) by NT-4

formation, chondrogenesis, and calcification. These data indicate that ALC is a useful experimental tool to analyze ameloblast character.

HERS01a cells were established from mouse molar [25]. Immunohistochemical staining and real-time PCR analysis showed that HERS01a cells expressed vimentin and N-cadherin as mesenchymal markers as well as cytokeratin14, E-cadherin, and p63 as epithelial stem cell markers. In periodontal development during root formation of a tooth, the epithelial-mesenchymal transition (EMT) has been a subject of controversy. Hertwig's epithelial root sheath (HERS), consisting of two epithelial layers, plays a role of inducing odontogenesis during root development and thereafter becomes fragmented. In the presence of TGF- β , HERS01a cells also expressed many more mesenchymal markers, as well as snail1 and 2 as EMT markers. Taken together, our data show that HERS01a displayed unique features associated with EMT in the root formation process, and will thus be useful for analyzing the biological characteristics of HERS and the molecular mechanism underlying the EMT.

3 Dental Epithelium from Odontogenic Tumor

To establish the cell lines, cells from tumor tissues were cultured for long time. Ameloblastomas are slowly growing, locally invasive neoplasms with a potentially destructive behavior. The molecular mechanisms that regulate the cell growth and invasion of ameloblastoma cells are unknown. Because ameloblastoma cells placed in culture have a very limited lifespan. Because of this slow proliferation, it is difficult to establish cell lines from odontogenic tumor, especially ameloblastoma and epithelial odontogenic tumor.

AM-1 cells were derived from human ameloblastoma using human papillomavirus type-16 [26]. This cell line maintains epithelial cell morphology and expresses cytokeratins K8, K14, K18, K19. Furthermore, bcl-2 protein, which prevents apoptosis, is expressed. These cells grew in a monolayer over foci of collagen

degradation and could invade the collagen gel at such sites. Since the behavior of cell line AM-1 mimics the behavior of ameloblastoma in vivo, it may be a valuable model for the study of these neoplasms.

HAM1, 2, 3 cells were established by the transfection of two retroviral constructs of human telomerase reverse transcriptase (hTERT) cDNA and mouse cyclin-dependent kinase 4 (cdk4) cDNA into the primary ameloblastoma cells [27]. Three cell lines were examined by electron microscopy, assay of senescence-associated β -galactosidase activity, mRNA expression and immuno-reactivity of dental epithelial marker cell molecules and enamel matrix proteins. They showed undifferentiated phenotypes in monolayer culture and did not have any β -galactosidase activity. The transcripts of dental epithelial cell markers of *Msx2*, *Jagged1*, *Notch1*, *Sp3*, *Sp6*, keratin 14 and keratin 18 were confirmed. In addition, mRNA and protein expression of ameloblastin and enamelin were also detected in three cell lines. All cells in the three cell lines were keratin 14- and 18-positive and some elongated cells were *Jagged1*-positive. *Msx2*-positive nuclei were noted in only HAM2 cells.

In the field of dental research, several kinds of dental epithelial cell lines with unique gene expression were established. Using these cells, molecular mechanism of ameloblast differentiation and progression of odontogenic tumors may be clearly identified.

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Metabolomic Approach to Oral Microbiota

Nobuhiro Takahashi, Jumpei Washio, and Gen Mayanagi

Abstract. Oral microbiota, known as dental plaque or oral biofilm, is a complex of microorganisms. Functions such as metabolic activity are crucial final outputs of microbiota, since they directly relate to the pathogenicity of oral diseases, such as dental caries and periodontitis. The metabolic characteristics of microbiota have been speculated from the results of in vitro studies of representative bacteria consisting of microbiota, because the amount of oral microbiota sampled from the oral cavity was too small for metabolic study. A new system of metabolome analysis (metabolomics), using the combination of capillary electrophoresis and a time-of-flight mass spectrometer, has been developed, enabling us to quantify hundreds of intracellular metabolic intermediates relevant to the central carbon metabolism in supragingival plaque. The metabolomic approach may offer new insights into understanding the metabolic activity of microbiota, as “a supra-organism” consisting of a tremendous number of organisms that may be considered a single organism.

Key words. Microbiota, Metabolome analysis, Metabolomics, Oral biofilm, Supra-organism

N. Takahashi (✉) and J. Washio

Division of Oral Ecology and Biochemistry, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: nobu-t@dent.tohoku.ac.jp

G. Mayanagi

Research Unit for Interface Oral Health Science, Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

1 Introduction

The term oral microbiota, often called dental plaque or oral biofilm, refers to the complex of microorganisms in the oral environment and their non-microbial surroundings. Research on oral microbiota is stratified as: (1) analysis of microbial composition, (2) analysis of microenvironments, and (3) analysis of the functions of microbiota [1–3]. Among these three research approaches, the functions of microbiota, such as metabolic activity, are crucial final outputs, since they directly relate to pathogenicity of oral diseases, such as dental caries and periodontitis.

Therefore, in this review, we focused on the metabolic systems of oral microbiota, particularly in supragingival plaque, and we introduced our recent metabolomic study of supragingival plaque as a method for metabolic analysis of microbiota in vivo.

2 Metabolic System of Oral Bacteria in Supragingival Plaque

There are a number of ecological niches in the oral cavity. These ecological niches can be characterized by environmental factors and the metabolic characteristics of the occupying microbiota. In supragingival plaque, environmental factors contribute as follows: the surface for microbial adhesion is the saliva-coated tooth surface; nutrition for bacteria is mainly saliva and sometimes carbohydrates derived from dietary foods; the pH is usually maintained as neutral, due to salivary flow; however, it sometimes becomes acidic when carbohydrates are supplied; the oxygen concentration is relatively high [1–3]. Under these conditions, saccharolytic microorganisms, in other words, carbohydrate-degrading bacteria, become dominant, such as *Streptococcus*, *Actinomyces* and *Lactobacillus*.

Figure 1 shows the central carbon metabolism, including the Embden–Meyerhof–Parnas pathway (the EMP pathway), the pentose-phosphate pathway and the tricarboxylic acid cycle (TCA cycle). Central carbon metabolism starts with classic glycolysis, the EMP pathway, in which glucose is degraded into pyruvate and, under anaerobic conditions, further degraded into lactate, formate and acetate by the catalysis of lactate dehydrogenase and pyruvate formate-lyase. These pathways are shared by *Streptococcus*, *Actinomyces* and *Lactobacillus* [4–6]. In the presence of oxygen, pyruvate is converted to acetate by the catalysis of pyruvate oxidase and pyruvate dehydrogenase in *Streptococcus* and *Lactobacillus*, and lactate is converted to acetate by the combination of lactate dehydrogenase and pyruvate oxidase in *Actinomyces*. In the presence of bicarbonate, abundantly contained in saliva, phosphoenolpyruvate is converted to succinate via oxaloacetate with bicarbonate assimilation by the catalysis of phosphoenolpyruvate carboxykinase and phosphoenolpyruvate carboxylase, using part of the TCA cycle, in *Actinomyces* [5, 6].

The production of acids, such as lactate, acetate, formate and succinate from carbohydrate, results in a pH decrease, which directly initiates dental caries.

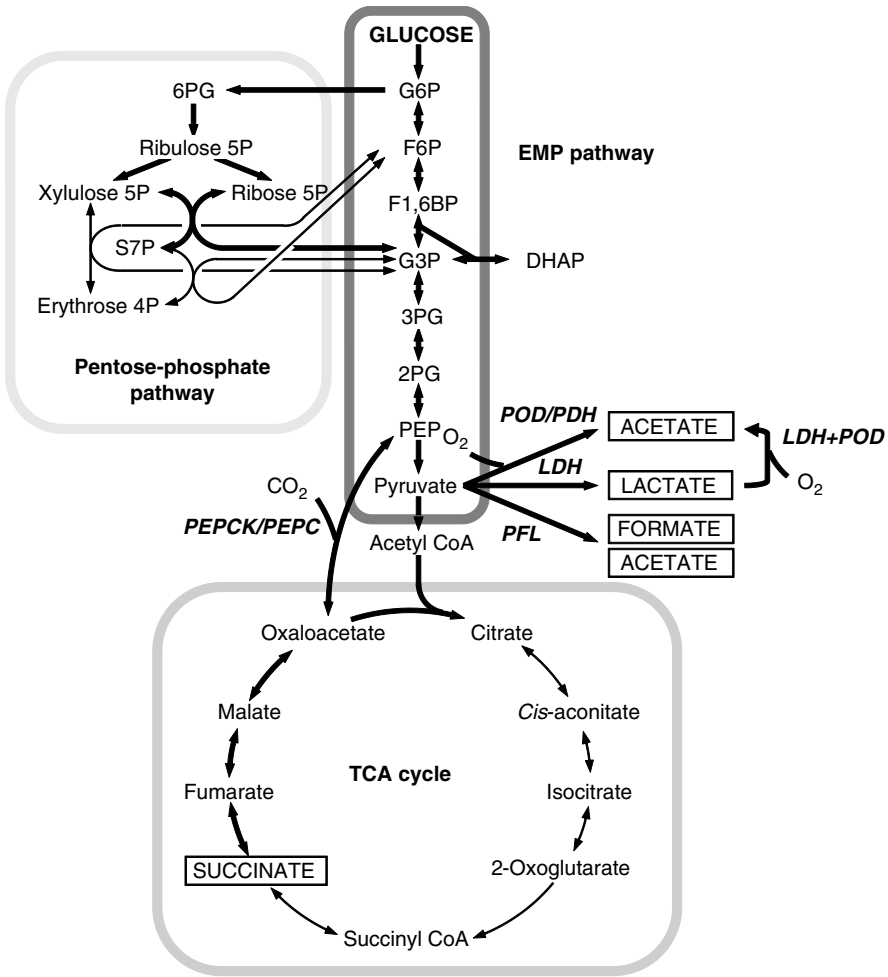


Fig. 1. Metabolic pathways of representative plaque bacteria. Metabolic intermediates: *G6P* glucose 6-phosphate, *F6P* fructose 6-phosphate, *F1,6BP* fructose 1,6-bisphosphate, *G3P* glyceraldehyde 3-phosphate, *DHAP* dihydroxyacetone phosphate, *3PG* 3-phosphoglycerate, *2PG* 2-phosphoglycerate, *PEP* phosphoenolpyruvate, *6PG* 6-phosphogluconate, *Ribulose 5P* ribulose 5-phosphate, *Ribose 5P* ribose 5-phosphate, *S7P* sedoheptulose 7-phosphate, *E4P* erythrose 4-phosphate. Metabolic enzymes: *LDH* FBP-dependent lactate dehydrogenase, *POD* pyruvate oxidase, *PDH* pyruvate dehydrogenase, *PFL* pyruvate formate-lyase, *PEPCK* phosphoenolpyruvate carboxykinase, *PEPC* phosphoenolpyruvate carboxylase

3 Supragingival Plaque pH In Vivo

pH monitoring in vivo is an excellent method to access the acid productivity (or metabolic activity) of supragingival plaque [7, 8]. A small pH electrode attached to a small piece of enamel is placed in the interproximal area of a denture and allowed

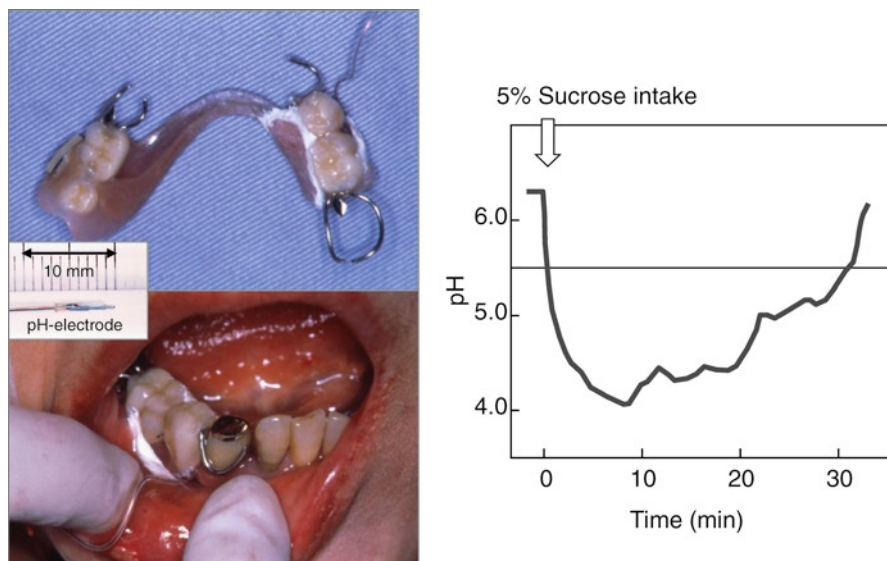


Fig. 2. Plaque pH telemetry system and an example of plaque pH in vivo after sucrose rinse

to accumulate dental plaque in the oral cavity of a volunteer (Fig. 2). The dental plaque pH is monitored telemetrically, so that it is possible to assess the metabolic activity of dental plaque in vivo. The pH measurement of supragingival plaque is informative about the metabolic activity of microbiota; however, it reflects only the total acidity of end-products of microbiota and thus is not enough for assessing the metabolic pathway and its regulation. In addition, the amount of oral microbiota sampled from the oral cavity is too small for a conventional metabolic study. In order to overcome this difficulty, metabolome analysis of oral microbiota is an excellent candidate.

4 Metabolomics of Supragingival Plaque Using CE-MS

Metabolome analysis, in other words, metabolomics, is the comprehensive identification and quantification of metabolites in biological systems, which is one of the most powerful approaches for metabolism research. Capillary electrophoresis combined with a mass spectrometer, CE-MS for short, has been developed, and has proven suitable to separate and quantify metabolites involved in the central carbon metabolism, since most metabolites are polar and ionic small molecules. Capillary electrophoresis is an excellent separator of ionized small biomolecules such as metabolic intermediates, and a time-of-flight mass spectrometer is an excellent analyzer of molecular mass [9–13].

Only about 10 mg supragingival plaque before and after an oral glucose rinse was sampled and applied to the CE-MS for the detection and quantification of

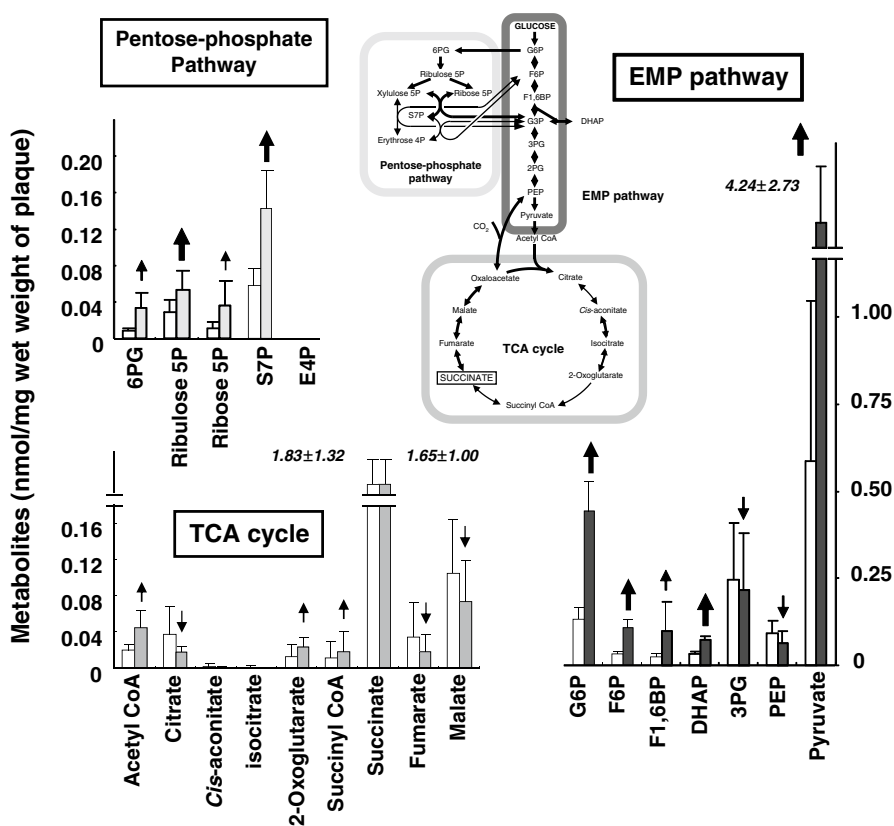


Fig. 3. Metabolomics of the EMP pathway, the pentose-phosphate pathway and the TCA cycle of supragingival plaque before and after glucose rinse. *Open box*, metabolites before glucose rinse; *closed box*, metabolites after glucose rinse; *vertical bar*, standard deviation. Significant increases after glucose rinse (*thick arrows*, $p < 0.002$ by *t*-test with Bonferroni correction for multiple comparisons). Increases and decreases after glucose rinse, although not significant (*thin arrows*). See the legend of Fig. 1 for abbreviations of metabolites

each metabolic intermediate of the central carbon metabolism, including the EMP pathway, the pentose-phosphate pathway and the TCA cycle (Fig. 3) [14]. After the glucose rinse, glucose 6-phosphate, fructose 6-phosphate, dihydroxyacetone phosphate and pyruvate in the EMP pathway, and ribulose 5-phosphate and sedoheptulose 7-phosphate in the pentose-phosphate cycle were found to have increased significantly. There were also many changes in metabolic intermediate levels after the glucose rinse, although these were not significant. Lactate was also increased from 1.74 ± 0.82 nmol to 13.1 ± 12.7 nmol per mg wet weight of plaque by glucose rinse. These data clearly show that we can profile metabolic intermediates in the central carbon metabolism even in a tiny amount of supragingival plaque.

In addition, metabolic intermediates in representative oral bacteria in supragingival plaque, *Streptococcus sanguinis*, *Streptococcus mutans*, *Actinomyces oris*, and *Acrinomyces naeslundii*, before and after glucose metabolism, were also measured by the CE-MS. The profile of metabolite change in supragingival plaque was basically similar to that obtained from a single bacterial strain [14]. These results suggest the novel idea that microbiota consist of a tremendous number of bacteria, but function as one organism, that is, “a supra-organism”. Furthermore, the data provide new information that microbiota in supragingival plaque utilize the pentose-phosphate pathway and most of the TCA cycle, as well as the EMP pathway, during glucose metabolism.

5 Conclusion

In conclusion, metabolome analysis enables us (1) to monitor the dynamic functions of metabolic activity in oral microbiota in vivo. In the near future, this method will be further applied (2) to elucidate drug efficacy in vivo, from classical cariostatic fluoride to new-generation antibiotic reagents, to oral microbiota, (3) to identify new biomarkers for oral diseases, and (4) to analyze host tissues and the host-parasite interface. The metabolomic approach may herald a new era in research on the function of microbiota and host tissues.

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Melastatin Transient Receptor Potential Channel Type 5

Minoru Wakamori, Takashi Yoshida, Takashi Kikuchi, Daisuke Kondoh,
and Masashi Komatsu

Abstract. Fat is one of the three major nutrients but it is not a sapid substance which activates one of the five basic tastes, sweet, bitter, sour, salt, and umami. However, we have experience that fat changes the taste of dishes largely. Transient receptor potential M5 (TRPM5) channel is not a receptor of the five basic tastes but it is gated after stimulation of the gustducin-coupled receptors for sweet, bitter, and umami. TRPM5 channel is a cation channel and depolarizes the taste cell, indicating that TRPM5 channel relates to the formation of tastes. The activities of some TRP channels (TRPV2, TRPV4, TRPM2, and TRPA1 channels) are regulated by lipids. I will review the TRP channels and taste transduction.

Key words. Channel, Taste bud, Transient receptor potential, TRP

M. Wakamori (✉), T. Yoshida, and T. Kikuchi
Laboratory of Molecular Pharmacology and Cell Biophysics, Department of Oral Biology,
Tohoku University Graduate School of Dentistry, Sendai 980-8575, Japan
e-mail: wakamori@dent.tohoku.ac.jp

D. Kondoh
Laboratory of Molecular Pharmacology and Cell Biophysics, Department of Oral Biology,
Tohoku University Graduate School of Dentistry, Sendai 980-8575, Japan
and
Laboratory of Operative Dentistry, Department of Restorative Dentistry,
Tohoku University Graduate School of Dentistry, Sendai 980-8575, Japan

M. Komatsu
Laboratory of Operative Dentistry, Department of Restorative Dentistry,
Tohoku University Graduate School of Dentistry, Sendai 980-8575, Japan

1 TRP Channels

Ca^{2+} controls diverse cellular processes, which include neurotransmitter release, gene expression, and cell proliferation [1, 2]. Multiple types of voltage-gated Ca^{2+} channels, and Ca^{2+} -permeable ligand-gated channels form major Ca^{2+} entry pathways in neurons. On the other hand, neither voltage-gated Ca^{2+} channels nor ligand-gated channels are expressed in non-excitabile cells including epithelial cells and lymphocytes. Stimulation of seven-transmembrane receptors that generates 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) from phosphatidylinositol-4,5-bisphosphate (PIP_2) via phospholipase C (PLC) elevates cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$), which is controlled by two components, IP_3 -induced Ca^{2+} release from intracellular Ca^{2+} stores, endoplasmic reticulum (ER), and Ca^{2+} influx across plasma membrane. Ca^{2+} influx is mediated by diverse Ca^{2+} -permeable ion channels activated by various triggers [3, 4]. An important clue for understanding the molecular basis of receptor-activated Ca^{2+} influx was first obtained through the finding of a *Drosophila transient receptor potential (trp)*, whose photoreceptors fail to generate the Ca^{2+} -dependent sustained phase of receptor potential and to induce subsequent Ca^{2+} -dependent adaptation to light [5, 6]. The *trp* proteins and its homologues form cation- and Ca^{2+} -permeable channels [7]. With regard to TRP homologues, so far 33 TRP proteins have been reported in mammals, and 28 TRP channels in human. They are subdivided into six subfamilies on the bases of sequence similarity, TRPM ('Melastatin') group, TRPC ('Canonical') group, TRPV ('Vanilloid') group, TRPP ('Polycystin'), TRPML ('Mucolipin'), and TRPA ('Ankyrin') [8]. The most familiar TRP channel is TRPV1, which is expressed in small and medium sized dorsal root ganglion (DRG) neurons and trigeminal ganglion (TG) neurons. Another name it is known by is capsaicin receptor. TRPV1 channel is activated in a polymodal manner by capsaicin, which is an irritant of hot pepper, as well as acid and noxious heat ($>43^\circ\text{C}$). Therefore, TRPV1 monitors extracellular environmental condition of the cells. Other TRP channels also monitor physical stimuli such as different temperatures (TRPV1, TRPV2, TRPV3, TRPV4, TRPM8 and TRPA1 channels) and osmo- (TRPC1, TRPC5, TRPC6, TRPV2, TRPV4, TRPM3 and TRPM7 channels) and mechano-stresses (TRPC1, TRPC6, TRPV1, TRPV2, TRPM4, TRPM7, TRPP2 and TRPA1 channels). Some TRP channels monitor chemical stimuli. TRPM2 channel is activated by micromolar levels of H_2O_2 and agents that produce reactive oxygen/nitrogen species, which is attributable to an agonistic binding of nicotinamide adenine dinucleotide ($\beta\text{-NAD}^+$) to the MutT motif in TRPM2 C-terminus [9]. TRPC5 channel is activated by extracellular Ca^{2+} [10], and is activated by nitric oxide through cysteine S-nitrosylation [11]. Therefore TRPM2 and TRPC5 channels monitor redox state of the cell. TRPM2 channel is also activated by arachidonic acid [9]. Because cations pass TRP channels, physical and chemical stimuli are converted into an electrical signal. In these cases that cells monitor extracellular conditions, TRP channels serve as signal amplifiers and converters as well as signal detectors (Fig. 1a).

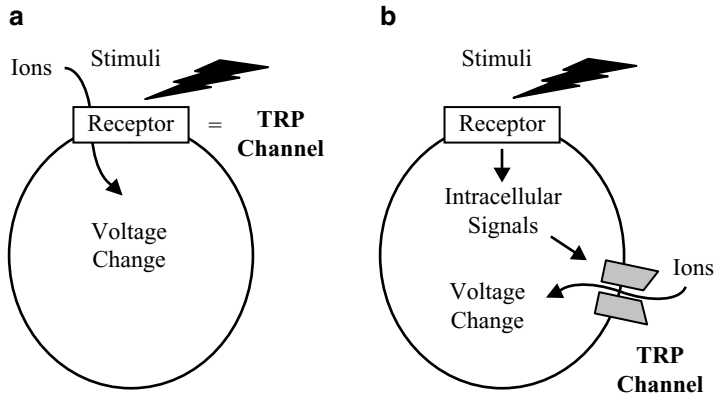


Fig. 1. Schematic models of voltage changes that TRP channels cause. **(a)** TRP channels are receptors of stimuli. TRP channels are permeable to cations which change membrane potential. **(b)** TRP channels aren't receptors of extracellular stimuli, but are activated by intracellular signals. Because TRPs are cation channels, TRP channels depolarize membrane

The first mammalian TRPM identified, TRPM1, was initially named melastatin, as its expression levels correlated inversely with the metastatic potential in some melanomic cell lines [12]. Based on similarities in amino acid sequences, 8 TRPM proteins fall into subsets consisting of TRPM1/3, TRPM4/5, and TRPM6/7. TRPM2 and TRPM8 are not placed in any subset. TRPM4 and TRPM5 have unique characteristics among the TRP superfamily in that they are voltage-dependent, Ca^{2+} -activated, and monovalent cation selective channels [13, 14]. Some TRP channels are activated by intracellular signals, calcium, PIP_2 , DAG [15]. In these cases that cells monitor extracellular conditions by the use of receptors which produce the intracellular signals. TRP channels serve as signal amplifiers and converters (Fig. 1b).

2 Taste Receptors and TRPM5 Channel

Sweet, umami, bitter, salty and sour are five basic tastes. Salty and sour substances activate channels, for example, the epithelial Na channel (ENaC), acid-sensing ion channel (ASIC) and polycystin kidney disease (PKD, TRPP) channels. On the other hand, umami, sweet, and bitter substances bind to seven-transmembrane receptors, G-protein coupled receptors. Two families of mammalian taste receptors, T1Rs and T2Rs, are implicated in sweet, umami and bitter tastes. After activation of G-protein coupled receptors, gustducin stimulates phospholipase C (PLC) $\beta 2$ which synthesizes IP_3 leading to the release of Ca^{2+} from intracellular stores. The released Ca^{2+} ion activates TRPM5 channels, which are permeated by positively charged Na^+ ions. Therefore, membrane depolarizes and consequently gates voltage-dependent Ca^{2+} channels. Increased intracellular Ca^{2+} ions trigger release of neurotransmitters.

Dietary lipids mainly consist of triglycerides, which are hydrolyzed into free fatty acids by the lingual lipase in the oral cavity. Free fatty acids inhibit the delayed rectifying potassium channels in rat taste receptor cells [16]. Interestingly, subset of the cells in circumvallate taste buds expresses the enzymes that can liberate arachidonic acid or metabolize arachidonic acid [17]. Unsaturated fatty acid, arachidonic acid, activates TRPV1, TRPV4 [15], TRPM2 channels [9] and TRPM5 [17], too.

The HEK cells were co-transfected with the plasmids containing cDNA for TRPM5 and the expression plasmid carrying EGFP, using Effectene Transfection Reagent (Qiagen). Forty eight hours after transfection, we selected the HEK cells expressing TRPM5 channels through detection of the marker GFP. The cells were subjected to the whole-cell mode of the patch-clamp technique. The calculated free Ca^{2+} concentration of the internal pipette solution with 5 mM EGTA and 1 mM MgCl_2 , 3.91 mM CaCl_2 was 0.5 μM . Current–voltage (I – V) relationships with the negative ramp pulse from +80 to –80 mV for 320 ms every 3 s were recorded. After formation of the whole-cell mode, free Ca^{2+} ions in the pipette solution entered the cell and activated the TRPM5 channel. After reaching the peak, current amplitude declined to the basal level. Then 1- μM ionomycin, Ca^{2+} ionophore activated the TRPM5 channel via an increase in $[\text{Ca}^{2+}]_i$ again. These I – V relationships have the hallmarks of the TRPM5 channel. The reversal potential is about 8 mV. The I – V relationship shows an outwardly rectifying behavior, not a linear regression. Because of lower free intracellular Ca^{2+} concentration (0.3 μM), activation of TRPM5 channel by internal perfusion of the cell was not observed. 10- μM of arachidonic acid induced outwardly rectifying current, of which reversal potential is about 5 mV. When intracellular Ca^{2+} was strongly chelated by 10-mM Bapta, arachidonic acid never induced any TRPM5 channel current. Therefore, we concluded that $[\text{Ca}^{2+}]_i$ is an activator of the TRPM5 channel but fatty acids are modulators of the TRPM5 channel. It is reported that pure fats are tasteless, but we have many experiences in which fats change the taste of foods. One of the mechanisms of this taste change may be the modulation of TRPM5 channel by fatty acids.

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Pannexin 3, a Gap Junction Protein, Regulates Chondrocyte Differentiation in Part Through Hemichannel Activity

Tsutomu Iwamoto, Mariko Ono, Makiko Arakaki,
Takashi Nakamura, Aya Yamada, and Satoshi Fukumoto

Abstract. Gap junctional communications play a crucial role for organogenesis including cartilage development. Pannexin 3 (Panx3) belongs to the new member of the gap junction pannexin family. However, the expression and function of Panx3 have not been cleared. Here, we demonstrate that Panx3 is expressed in cartilage, and regulates chondrocyte differentiation in vitro cell culture. Our observations indicate that Panx3 plays a crucial role for the differentiation of chondrocyte, and suggest that Panx3 is a key molecule for cartilage development.

Key words. Cartilage, Gap junction, Pannexin

1 Introduction

Cartilage is a highly specialized connective tissue, and has important roles for the formation of two critical skeletal elements: the growth plate and the articular cartilage. The majority of bones in the body are formed through the process of endochondral ossification. The endochondral ossification is initiated by the condensation of mesenchymal progenitor cells. Then, the mesenchymal cells differentiate into chondrocyte. The growth plate is comprised of the chondrocyte, and is arranged in a columnar fashion in proliferation and differentiation processes, which define the length of bone. The articular chondrocytes forms a chondron, consists of a small number of chondrocytes, and are responsible for the synthesis and maintenance of the extracellular matrix. The articular cartilage has a role for a shock absorber in synovial joints.

T. Iwamoto (✉), M. Ono, M. Arakaki, T. Nakamura, A. Yamada, and S. Fukumoto
Division of Pediatric Dentistry, Department of Oral Health and Development Sciences,
Tohoku University Graduate School of Dentistry, 4-1 Seiryō-machi, Aoba-ku,
Sendai 980-8575, Japan
e-mail: iwamoto@dent.tohoku.ac.jp

Gap junctions are intercellular channels that allow the movement of small molecules including ions, nucleotides, and second messengers, for the direct cell-to-cell communication between the adjacent cells. In cartilage development, connexin 43 (Cx43) appears in the mesenchymal condensations of early developmental stage, and is more restricted to the presumptive area for perichondrium [1]. In particular, Cx43 is important in cell signaling processes involved in limb growth and patterning. Cx43 is also detected in the paired chondrocytes of the superficial zone of articular cartilage [2]. So far, it had been thought that typical gap junctions are not developed between the columnar chondrocytes of the growth plate, because the cells are surrounded by a large amount of extracellular matrix.

Pannexins, consist of three member: pannexin 1 (Panx1), pannexin 2 (Panx2), and pannexin 3 (Panx3), are recently identified as a second gap junction family. Both Panx1 and Panx2 are preferentially expressed in the central nervous system. However, the expression and the physiological function of Panx3 had not been clearly elucidated.

2 Material and Methods

Frozen tissue sections from growth plates (E16.5) were generated and placed on RNase-free glass slides for in situ hybridization. ATDC5 cells and N1511 cells were used for in vitro studies. All experiments were performed by the methods that described previously [3].

3 Results and Discussion

In situ hybridization revealed that the expression of Panx3 was observed in prehypertrophic chondrocytes. Overexpression of Panx3 promoted the differentiation of chondrocyte cell lines, ATDC5 and N1511. Conversely, the suppression of endogenous Panx3 by shRNA inhibited differentiation of these cells. Interestingly, Panx3-transfected cells reduced parathyroid hormone-induced cell proliferation. Further, Panx3 promoted the release of ATP into the extracellular space. As the results, intracellular cAMP levels and the activation of cAMP-response element binding were reduced. These results suggest that the functions of Panx3 are to switch the chondrocyte cell fate from proliferation to differentiation by regulating the intracellular ATP/cAMP levels in part through its hemichannel activity. Thus, Panx3 is likely the major gap junction protein in growth plate cartilage.

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Positive Effect of Whole-Body Vibration Loading on Peri-Implant Bone Healing and Implant Osseointegration

Toru Ogawa, Xiaolei Zhang, Ignace Naert, Keiichi Sasaki,
and Joke Duyck

Abstract. The aim of this study was to evaluate the effect of low-magnitude, high-frequency (LMHF) loading, applied by means of whole body vibration (WBV), on peri-implant bone healing and implant osseointegration in rat tibiae.

A custom-made titanium implant was inserted in the proximal metaphysis of the tibiae of male Wistar-rats. Animals were divided to unloaded control group and several test (loaded) groups received LMHF WBV loading with different loading parameters. Histological assessment was done combined with histomorphometrical measurements. Bone-to-implant contact (BIC) and peri-implant bone fraction (BF) were analyzed for histomorphometrical measurement.

It was confirmed the bone stimulating potential of LMHF loading on peri-implant bone healing and implant osseointegration. Furthermore, time of loading significantly influenced the effect of the WBV on peri-implant bone healing. Twice short periods of loading appears to have the most favorable effect. In addition, loading parameters such as frequency and acceleration significantly influenced to the effect on peri-implant bone.

T. Ogawa (✉)

Department of Prosthetic Dentistry, BIOMAT Research Cluster,
Katholieke Universiteit Leuven, Leuven, Belgium
and

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: ogat-thk@umin.ac.jp

X. Zhang, I. Naert, and J. Duyck

Department of Prosthetic Dentistry, BIOMAT Research Cluster,
Katholieke Universiteit Leuven, Leuven, Belgium

K. Sasaki

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

Key words. Low-magnitude and high-frequency loading, Oral implant, Peri-implant bone, Rat tibia, Whole body vibration

1 Introduction

It is observed that low-magnitude, high-frequency (LMHF) loading can have an osteogenic effect to bone [1–3]. The aim of this study was to evaluate the effect of LMHF loading, applied by means of whole body vibration (WBV), on peri-implant bone healing and implant osseointegration in rat tibiae.

2 Method of Animal Experiments

A custom-made titanium implant was inserted in the proximal metaphysis of the tibiae of male Wistar rats. Animals were divided to unloaded control group and several test (loaded) groups received LMHF WBV loading with different loading parameters. The LMHF loading was applied through WBV. A custom-made WBV device was used (Department of Mechanical Engineering, Division of Biomechanics and Engineering Design, K. U. Leuven). Histological assessment was done combined with histomorphometrical measurements. Bone-to-implant contact (BIC) and peri-implant bone fraction (BF) were analyzed for histomorphometrical measurement.

3 Effect of LMHF Loading on Peri-Implant Bone Healing in Rats [4]

Histological data from 7-day healed implants clearly showed an osteogenic reaction around the implants, particularly in the test group. After 25 days of healing, the peri-implant bone appeared to be much denser. Mean BIC and BF were significantly influenced by both the loading and the healing time ($p < 0.01$, ANOVA). The results of this study confirm the bone stimulating potential of LMHF loading on peri-implant bone healing and implant osseointegration.

4 Influence of LMHF Loading Time on Peri-Implant Bone Healing [5]

The rats were divided into five groups with different loading times: 0 (control/non-loading), 1.25, 2.5, 5 and twice 1.25 min (with an interim recovery period) of loading. Time of loading significantly influenced the effect of the WBV on peri-implant bone healing. Twice 1.25 min of loading appears to have the most favorable effect.

5 Effect of Frequency and Magnitude of LMHF Loading on Peri-Implant Bone Healing

The rats were divided into six groups: a control group (no loading) and five test groups with different loading frequency ranges and accelerations (12–30 Hz at 0.3 g; 70–90 Hz at 0.3 g; 70–90 Hz at 0.075 g; 130–150 Hz at 0.3 g; and 130–150 Hz at 0.043 g). BIC of each test group was significantly higher than that of the control group for both healing periods. Loading frequency and acceleration significantly influenced BIC and BF (ANOVA: $p < 0.01$). The 130–150 Hz (0.3 g) loading group demonstrated the most favorable effect. This result suggests both frequency and magnitude (acceleration) might be influenced to vibration effect on peri-implant bone healing. The tendency which the higher frequency and magnitude, the more favorable effect is observed.

6 Conclusion

The results of these studies confirm the bone stimulating potential of LMHF loading on peri-implant bone healing and implant osseointegration. There are many loading parameters such as loading time (duration and sequence etc.), loading magnitude or frequency that affect the loading effect. An appropriate LMHF loading regime can improve and accelerate peri-implant bone healing and osseointegration.

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Calcium Phosphate-Coated Titanium Alloy Implants Prepared by Radiofrequency Magnetron Sputtering: A Review

Naru Shiraishi, Yuko Suzuki, Naoko Sato, Takahisa Anada, Takashi Goto, Rong Tu, Mitsuo Niinomi, Takayuki Narushima, Kyosuke Ueda, Risa Uzuka, Osamu Suzuki, and Keiichi Sasaki

Abstract. This review summarized dental implant coated with calcium phosphate films, especially prepared by radiofrequency (RF) magnetron sputtering system. Calcium phosphate films on titanium alloys fabricated by RF magnetron sputtering system have very thin layer (within 500 nm), high bonding strength (over 60 MPa) and excellent biocompatibilities, even though its films created under the condition of room temperature. This method may be providing the effective interface between tissues and some other materials.

Key words. Calcium phosphate coated implant, Interface, Osseointegration, Radiofrequency magnetron sputtering system, Ti-29Nb-13Ta-4.6Zr alloys (TNTZ)

N. Shiraishi (✉)

Division of Craniofacial Function Engineering, Tohoku University Graduate School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan and

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan
e-mail: nshiraishi@dent.tohoku.ac.jp

Y. Suzuki, N. Sato, R. Uzuka, and K. Sasaki

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

T. Anada and O. Suzuki

Division of Craniofacial Function Engineering, Tohoku University Graduate School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

T. Goto, R. Tu, and M. Niinomi

Institute for Materials Research, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan

T. Narushima and K. Ueda

Department of Materials Processing, Tohoku University, 6-6-2 Aoba, Aramaki, Aoba-ku, Sendai 980-8579, Japan

1 Introduction

Titanium (Ti) and titanium alloys have been widely used as biomaterials because of their excellent biocompatibility [1–3]. Therefore, in dental treatments, implant treatment has become a major reconstructive method for the edentulous sites. Osseointegration, direct contact between implant and living bone, is essential phenomenon found in titanium–bone interface. It is known that titanium coated with calcium phosphate leads to early osseointegration. However, destroying the coating film and the detachment between implants and the film have been reported, which might affect long term prognosis [4, 5]. The present article was focused on the effects of calcium phosphate films created with radiofrequency (RF) magnetron sputtering system.

2 Preparation of Calcium Phosphate Films

The methods of coating calcium phosphate films on titanium, such as plasma spraying, sputtering, sol–gel and electrophoretic deposition, were reported. Among these methods, plasma spraying is widely used, because of its high deposition rates. However, the calcium phosphate films by plasma-spraying demonstrate poor adherence to the metal substrate [4, 5]. The high heating temperature of the substrates in the process of deposition may be contributing to its failures. Narushima et al. previously reported that amorphous calcium phosphate (ACP) films were fabricated using a RF magnetron sputtering system on Ti and Ti-6Al-4V alloy under the condition of room temperature. ACP films fabricated by this method have very thin layer (within 500 nm) and high bonding strength (over 60 MPa) [6, 7].

3 Evaluation of ACP Films Fabricated by RF Magnetron Sputtering System

To assess of biocompatibility with bone, specimen was inserted in mandibles of beagle dogs and femurs of rabbit. The bone-implant contact of ACP-coated commercially pure Ti implants was significantly higher than that of non-coated groups [7]. Moreover, removal torque value of ACP-coated Ti-6Al-4V implants had significantly higher values than the non-coated [8].

4 The Future Research in Our Group

On receiving these reports [6–8], we will apply the ACP films on Ti-29Nb-13Ta-4.6Zr alloys (TNTZ). TNTZ has excellent advantages including low Young's modulus (around 60 GPa), very low cytotoxicity similar to that of Ti and not having allergic elements [9]. However, disadvantage is the poor ability to form osseointegration with bone as compared with other bioactive Ti alloys. To improve biocompatibility of TNTZ, we will design the experiment to fabricate the ACP films on TNTZ by RF magnetron sputtering system and investigate the effects of this surface modification. This method might be improved the biocompatibility, biomechanical behavior and bioactivity of TNTZ.

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Effects of CO₂ Laser Irradiation of the Gingiva During Tooth Movement

Masahiro Seiryu, Toru Deguchi, Koji Fujiyama, Yuichi Sakai, Takayoshi Daimaruya, and Teruko Takano-Yamamoto

Abstract. It was hypothesized that CO₂ laser irradiation may reduce the early responses to nociceptive stimuli during tooth movement. The distribution of Fos-immunoreactive (Fos-IR) neurons in the medullary dorsal horn of rats was evaluated. Two hours after tooth movement, Fos-IR neurons in the ipsilateral part of the medullary dorsal horn increased significantly. CO₂ laser irradiation to the gingiva just after tooth movement caused a significant decrease of Fos-IR neurons. PGP 9.5- and CGRP-positive nerve fibers were observed in the PDL of all study groups. It was suggested that CO₂ laser irradiation reduced the early responses to nociceptive stimuli during tooth movement and might not have adverse effects on periodontal tissue.

Key words. c-Fos, CO₂ laser, Medullary dorsal horn, Rat, Tooth movement

Patients often feel pain or discomfort in response to orthodontic force. Pain from orthodontic treatment is mostly local, and therefore may be controlled more efficiently by locally administered analgesic treatment. One suggested method to control pain is laser therapy. In the present study, it was hypothesized that CO₂ laser irradiation could reduce the early responses to nociceptive stimuli during tooth movement.

The distribution of Fos-IR neurons in the medullary dorsal horn of rats was evaluated. Furthermore, to evaluate the nerve injury, we examined the distribution of

M. Seiryu, T. Deguchi, Y. Sakai, T. Daimaruya, and T. Takano-Yamamoto (✉)
Division of Orthodontics and Dentofacial Orthopedics, Tohoku University Graduate
School of Dentistry, 4-1, Seiryomachi, Aoba-ku, Sendai, 980-8574, Japan
e-mail: t-yamamo@m.tohoku.ac.jp

K. Fujiyama
Fujiyama Orthodontic Clinic Kyoto, Kyoto, Japan

protein gene product (PGP) 9.5-positive nerve fibers, which visualizes the entire tissue innervation, and the distribution of CGRP-positive nerve fibers, which are involved in periodontal tissue remodeling.

Forty 7-week-old male Wistar rats were used. The experimental animals were randomly divided into five groups: control group, mouth-open group, laser irradiation group, tooth-movement group, and tooth movement with laser irradiation group. Tooth-movement group rats were anesthetized and a piece of orthodontic elastic module was inserted between the maxillary first and second molars on the right side. The laser was administered to the buccal and palatal gingiva on the right maxillary molars for 15 s each.

The number of Fos-IR neurons on the ipsilateral side of the medullary dorsal horn was markedly higher in the tooth-movement group compared with the control group (Figs. 1e and 2); however, significantly lower numbers of Fos-IR neurons on the ipsilateral side of the medullary dorsal horn were observed in the tooth-movement with laser-irradiation group as compared with in the tooth-movement group (Figs. 1g and 2). PGP 9.5 and CGRP-positive nerve fibers were observed in the PDL of all study groups. The maximum temperature below the mucosa during CO₂ laser irradiation was less than 40°C.

CO₂ laser irradiation in this study significantly reduced the number of Fos-IR neurons 2 h after tooth movement in rats, thereby demonstrating that CO₂ laser irradiation reduced the early responses to nociceptive stimuli during tooth movement. Furthermore, CO₂ laser irradiation as used in this study might not have adverse effects on the periodontal tissue.

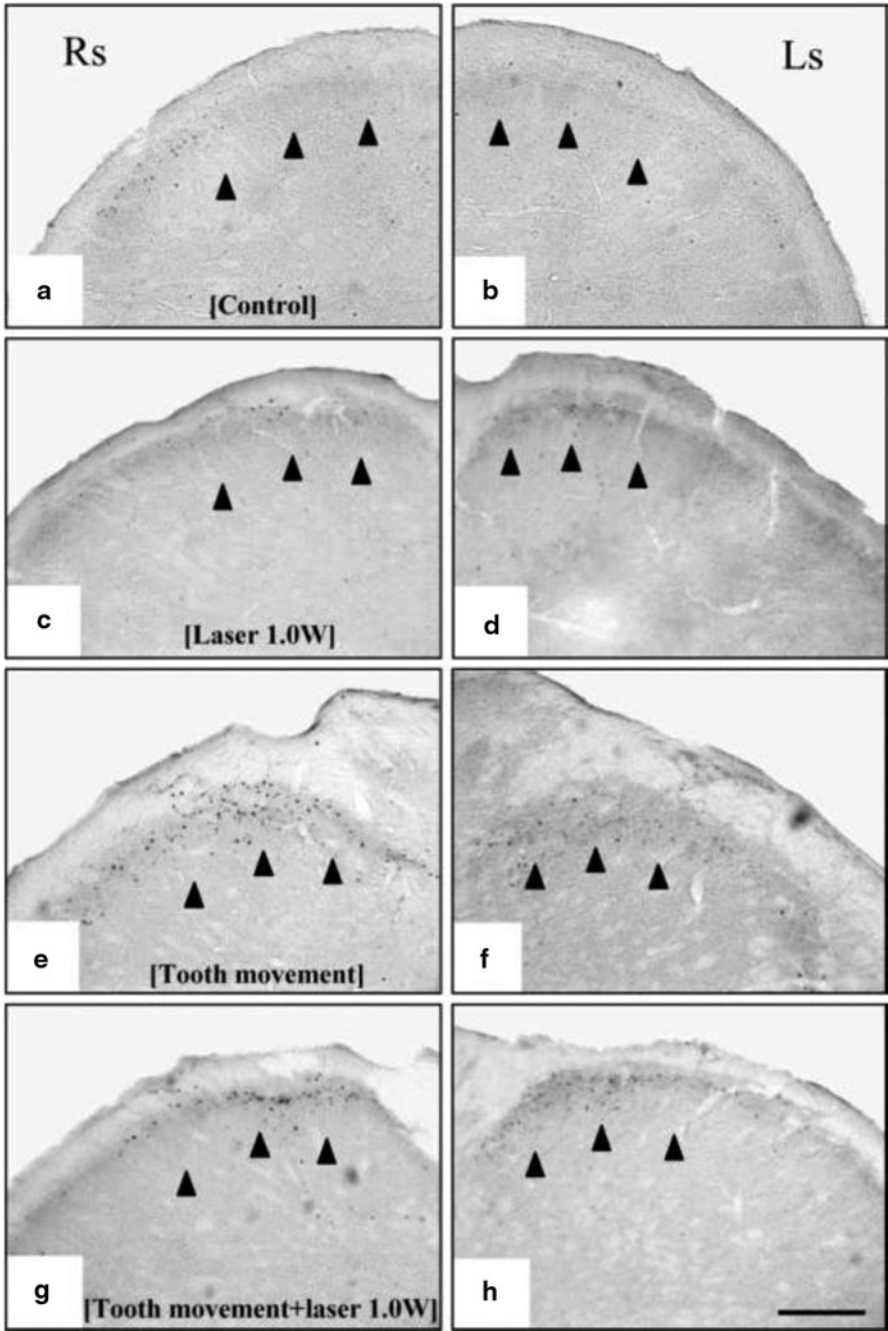


Fig. 1. Photomicrographs of Fos-IR neurons in the medullary dorsal horn at the level of the obex 2 h after the experimental treatment (a, c, e, g, ipsilateral side; b, d, f, h, contralateral side). Reproduced from Seiryu et al. [1]

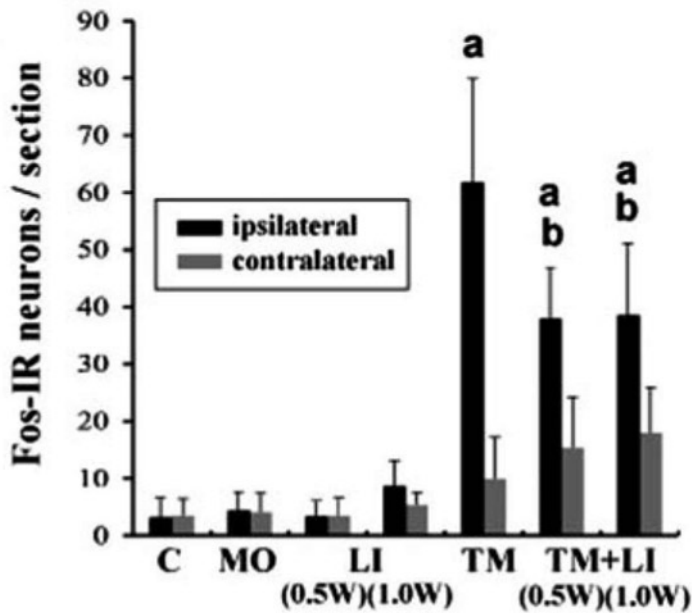


Fig. 2. The number of Fos-IR neurons on the ipsilateral and contralateral sides of the medullary dorsal horn. Reproduced from Seiryu et al. [1]

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Regulation of Airway Responsiveness by Dopamine Receptor Signaling Pathways

Kentaro Mizuta

Abstract. In this chapter, we have demonstrated that dopamine receptor subtypes are expressed on airway smooth muscle (ASM) and modulates ASM function. We detected the expression of dopamine D₁ and D₂ receptors in ASM. Dopamine D₁ receptor agonist stimulated cAMP production in human ASM cells and dose-dependently relaxed guinea pig ASM. Acute activation of the dopamine D₂ receptor in human ASM cells inhibited forskolin-stimulated adenylyl cyclase activity. On the other hand, chronic activation of the dopamine D₂ receptor stimulated it, and potentiated forskolin-induced guinea pig ASM relaxation. These findings suggest that functional dopamine D₁ and D₂ receptors are expressed in ASM, and modulate ASM tone through adenylyl cyclase/cAMP signaling.

Key words. Adenylyl cyclase, Airway smooth muscle, Asthma, Dopamine

1 Introduction

Dopamine receptors are G protein-coupled and comprise two subgroups, D₁-like receptors (D₁ and D₅), which couple to G_s protein and D₂-like receptors (D₂, D₃ and D₄), which couple to G_i. [1]. In patients with asthma, the administration of dopamine, systemically or topically, has a bronchodilatory effect in asthmatic subject [2, 3]. However, functional expression of the dopamine receptor subtypes has never been described on airway smooth muscle (ASM) itself.

K. Mizuta (✉)

Department of Dento-oral Anesthesiology, Tohoku University Graduate
School of Dentistry, 4-1 Seiryō-machi, Aoba, Sendai, Miyagi 980-8575, Japan
e-mail: mizuta@m.tohoku.ac.jp

2 Expression of Dopamine Receptor in Airway Smooth Muscle

The dopamine receptors are ubiquitously expressed in the central nervous system as well as peripheral and non-neuronal cells such as pulmonary artery [4]. Recently, we detected the mRNA encoding the dopamine D₁, D₂ and D₅ receptors isolated from native human ASM tissue and cultured ASM cells. The dopamine D₁ and D₂ receptors protein were also identified in human ASM tissue, cultured ASM cells and guinea pig ASM tissue by immunoblots and immunohistochemistry.

3 Modulation of Airway Smooth Muscle Tone Through Dopamine Receptor

Activation of G_s-coupled receptors leads to stimulation of adenylyl cyclase activity and a consequent increase in cellular cyclic AMP (cAMP) levels, which promotes ASM relaxation [5]. In contrast, the stimulation of G_i-coupled receptors leads to inhibition of adenylyl cyclase activity and a consequent reduction in cellular cAMP levels [6], which classically impairs ASM relaxation [7]. However, when the G_i-coupled receptors are chronically activated, there is a paradoxical and perhaps compensatory enhancement of adenylyl cyclase activity, which is called heterologous sensitization [8]. This sensitization has been demonstrated following the persistent activation of a number of G_i-coupled receptors, including dopamine D₂ receptor [8]. In our findings, activation of dopamine D₁ receptor on airway smooth muscle stimulated cAMP production through adenylyl cyclase/cAMP accumulation. As for dopamine D₂ receptor, acute activation of the dopamine D₂ receptor expressed on ASM contracted the ASM through adenylyl cyclase/cAMP inhibition, while chronic activation of this receptor relaxed the muscle through heterologous adenylyl cyclase/cAMP sensitization.

In conclusion, functional dopamine D₁ and D₂ receptors are expressed in ASM, and modulate ASM tone through adenylyl cyclase/cAMP signaling. These findings suggest a novel approach for functional relaxation of ASM through the dopamine receptor.

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Influence of Loading on Bone Metabolism Around Dental Implants in Rat Tibia

Miou Yamamoto, Masayoshi Yokoyama, Shigeto Koyama, Hiroto Sasaki, Yoshihito Funaki, Youhei Kikuchi, Hiromichi Yamazaki, Keizo Ishii, and Keiichi Sasaki

Abstract. This article reviewed the bone metabolic activity around titanium implants in rat tibia under the mechanical loading condition on the implant.

Key words. Bone metabolism Dental implant, Mechanical loading, PET scanner

M. Yamamoto (✉), M. Yokoyama, H. Sasaki, and K. Sasaki
Division of Advanced Prosthetic Dentistry, Tohoku University Graduate
School of Dentistry, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: miyamamoto@dent.tohoku.ac.jp; miyamamoto-thk@umin.net

S. Koyama
Maxillofacial Prosthetics Clinic, Tohoku University Hospital,
Sendai 980-8574, Japan

Y. Funaki
Division of Radiopharmaceutical Chemistry,
Cyclotron and Radioisotope Center, Tohoku University, Sendai, Japan

Y. Kikuchi
Division of Quantum Science and Energy Engineering,
Tohoku University Graduate School of Engineering, Sendai, Japan

H. Yamazaki
Division of Radiation Protection and Safety Control,
Tohoku University Cyclotron Radioisotope Center, Sendai, Japan

K. Ishii
Division of Radiation Protection and Safety Control,
Tohoku University Cyclotron Radioisotope Center, Sendai, Japan
and
Division of Quantum Science and Energy Engineering,
Tohoku University Graduate School of Engineering, Sendai, Japan

1 Introduction

Mechanical load onto the dental implant is thought to be one of the major factors affecting the condition of bone-implant interface. There is a general understanding that a certain level of mechanical loading is required for normal bone remodeling. On the other hand, it is suggested that excessive loading might lead to disintegration of bone-implant interface. It is not certain, however, how the loading influences on the bone metabolism around the dental implant. Although a lot of biomechanical and biological studies about loaded implants have been reported, there is a few knowledge of cross-sectional observation with application of static force. Moreover, there is little longitudinal study. This paper, therefore, aimed to review the influence of loading on dynamic changes of bone metabolism around dental implants by using a newly developed high resolution PET scanner (Tohoku university, Sendai, Japan) [1].

2 Quantitative Evaluation of Bone Metabolic Activity by Using PET Scanner

The bone metabolic activity was evaluated around the titanium implant, which was inserted in the tibiae of 12 weeks Wistar rat, at 13 mm distal from another implant placed 5 mm from the knee joint. For the mechanical loading to the implants, the coil springs were attached to implant heads 1 day after implant insertion. The PET scanner images of the rats with ^{18}F fluoride ion ($^{18}\text{F}^-$) (5 mCi/rat) intravenously-injected were taken by a practical semiconductor animal PET scanner at 4, 7, 14, 28 days after loading. The accumulation of $^{18}\text{F}^-$ of region of interest (ROI), defined around distal implant and intact area of opposite tibiae were evaluated as the activity level of bone metabolism. Micro CT images were also obtained and used for the fusion images with PET images to identify the activated regions in relation to the loaded implant.

3 Bone Metabolic Activity Around Implants Induced by Load Application

The accumulation counts around the implants gradually increased in initial 7 days, which peak level reached to 4 times of the intact control area, and then decreased with time and eventually became same level as the intact control. These phenomena suggest that activation of bone metabolism by application of static load in some extent is transient. Additionally, higher activities were shown at cancellous bone than at cortical bone. When four rectangular ROIs were set to compare the regional activities around the implants, the superior part of traction side and the inferior part of opposite side showed significantly higher accumulation counts compared with those of other parts at 7 days.

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The Forsyth Institute

Matrix Metalloproteinase-20 and Ameloblast Cell Movement in Rows

John D. Bartlett

Abstract. Ameloblasts move in rows that slide by one another to form the decussating enamel rod pattern characteristic of rodent teeth. Each rod is formed by one ameloblast and each rod preserves a complete record of the migratory path of the ameloblast that formed it. *Mmp20* null mouse enamel has at best a grossly malformed rod pattern and the maturation stage ameloblasts of the enamel organ overlap and grow atop one another. This suggests that ameloblast cell movement is restrained and that cell–cell attachment is amplified in the *Mmp20* null mouse enamel organ. Cadherins are a family of proteins that span the ameloblast cell membrane mediating attachment to identical cadherins present on adjacent ameloblasts. Herein we postulate that MMP20 cleaves the extracellular domains of ameloblast cadherins to allow ameloblasts to move synchronously in rows to form the characteristic and complex decussating enamel rod patterns.

Key words. Ameloblast, Cadherin, Enamel, MMP20, Motility, Movement

1 Introduction

Little is known about how the ameloblasts of the enamel organ attach, detach, move, and communicate with one another. Ameloblasts move in rows during the secretory stage of development and this movement culminates in the characteristic decussating enamel prism pattern [1–8]. However, the mechanism(s) responsible for controlling their motions remains a mystery. Below is a brief overview of enamel formation and how adherens junctions and MMPs are essential for guiding cell movement.

J.D. Bartlett (✉)
Department of Cytokine Biology, The Forsyth Institute,
245 First Street, Cambridge, MA 02142, USA
e-mail: jbartlett@forsyth.org

1.1 Overview of Dental Enamel Development

Dental enamel development progresses through defined stages that can be observed by the changing morphology of the enamel organ covering the developing tooth [9]. The ameloblasts of the enamel organ are a single layer of tall, columnar cells responsible for enamel development. When ameloblasts progress to the secretory stage, they elongate and form Tomes' processes at their apical ends nearest the forming enamel. This is when proteins including MMP20 are secreted from the Tome's processes into the enamel matrix and when long thin crystallites form normal to the secretory surface of the ameloblasts. The crystallites are bundled into rods or they are found between the rods, and each rod is the mineralized trail left behind by one ameloblast [10, 11]. Rows of ameloblasts will move laterally in alternating directions to form complex decussating rod patterns such as those seen in rodent incisor enamel [5–7]. Once the enamel layer reaches its full thickness, the ameloblasts retract their Tomes' processes and transition (transition stage) into shortened maturation stage cells. It is during the maturation stage that ameloblasts actively remove the bulk of the previously secreted proteins from the enamel layer as the crystallites grow in width and thickness (reviewed in [12, 13]). Therefore, ameloblasts progress through defined developmental stages that require intercellular contact and communication.

1.2 Overview of Adherens Junctions

Adherens junctions (AJ) perform multiple functions including: initiation and stabilization of cell–cell adhesion, regulation of the actin cytoskeleton, intracellular signaling and transcriptional regulation. The AJ is composed of several classes of protein including cadherins and catenins. Cadherins are transmembrane proteins with extracellular domains that provide important adhesive contacts between neighboring cells. The extracellular domains connect through homotypic trans-pairing between cadherins on adjacent cells and the intracellular domains of cadherins are linked to the actin cytoskeleton by the catenins (reviewed in [14]). p120-catenin (p120) binds to an intracellular cadherin domain near the cell membrane termed the “juxtamembrane domain” (JMD). Binding of p120 to the JMD prevents cadherins from becoming internalized and degraded [15–17]. Previously, we demonstrated that targeted ablation of p120 in epithelial tissues severely disrupted enamel development in mice [18]. Therefore, p120 and the cadherins it stabilizes are essential for dental enamel development.

α - and β -catenin are also part of the AJ. They link the actin cytoskeleton to cadherins. Rho GTPases regulate cytoskeletal structure and function [19] and cleavage of E-cadherin by MMP7 activates RhoA and promotes cell proliferation [20]. A major pathway for signal transduction by AJs involves regulation of β -catenin, which can act as either a structural protein at cell–cell junctions or a transcription factor in the cell nucleus. Disruption of AJs releases β -catenin for subsequent

degradation or translocation to the nucleus (reviewed in [21]). It was demonstrated previously that when MMPs cleave an extracellular cadherin domain, β -catenin is removed from its position near the cell membrane and in many cases will translocate to the cell nucleus [20, 22–27]. This translocation is associated with cell movement. Interestingly, when epithelial cells migrate within a tissue, their cell–cell junctions are disrupted and the actin cytoskeleton is reorganized. This is when cell surface E-cadherin is down-regulated and cell surface N-cadherin is up regulated [28].

2 MMP20 and Ameloblast Cell Movement in Rows

2.1 The Matrix Metalloproteinase-20 (*Mmp20*) Null Mouse

MMPs are a family of proteinases capable of cleaving virtually all extracellular matrix proteins including the extracellular domain of cadherins. MMPs play critical roles in reproduction, development, morphogenesis, wound healing, tissue repair, regeneration, remodeling, and cell movement (reviewed in [29]). MMP20 is required for healthy dental enamel development. People and mice with homozygous *MMP20* mutations have soft discolored enamel that may be hypoplastic and easily abrades from the dentin surface [30–36]. Analyses of *Mmp20* null mouse enamel revealed that the most abundant enamel matrix protein, amelogenin, is not processed properly, that the enamel has altered or non-existent rod patterns, and that the enamel organ has a deteriorating morphology as enamel development progresses [32]. Strikingly, the *Mmp20* null mouse enamel organ morphology is noticeably dysplastic during the maturation stage of development when MMP20 is no longer expressed. We theorize that an inability to cleave cadherins results in tight ameloblast cell–cell attachments contributing to the maturation-stage enamel organ dysplasia. We also theorize that the tight ameloblast attachments preclude ameloblast movement necessary to form the decussating enamel rod pattern.

2.2 MMP20 and Ameloblast Cell Movement

MMPs can initiate cell movement through cadherin hydrolysis. Previously, E-cadherin was demonstrated to inhibit cell surface localization of the pro-migratory 5T4 oncofetal antigen in mouse embryonic stem cells thereby inhibiting cell motility [37]. In contrast, MMP-3, -7, -9 cleave the extracellular domain of E-cadherin to promote cell movement, cell invasion, and/or cell proliferation [20, 24–26, 38]. Additionally, MMP-2, -9, -12, and -28 also cleave the extracellular domains of various cadherins [27, 39, 40]. Ameloblasts express E-, N- and P-cadherins, β -catenin and p120-catenin during enamel development [18, 41–46].

Significantly, adherens junctions have been identified along the possible sliding interface of adjacent secretory stage ameloblast migrating rows [47]. We expect that MMP20 promotes ameloblast movement necessary to form the decussating enamel rod pattern because we have recently demonstrated that MMP20 cleaves the E-cadherin extracellular domain [48].

3 Summary

We know that MMPs cleave cadherins to promote cell movement and we know from the malformed rod pattern in *Mmp20* null mice that the ameloblasts do not migrate properly. Also, the ameloblast dysplasia observed in the null mouse enamel organ is consistent with a failure of ameloblasts to detach from one another. We therefore expect that MMP20 cleaves the extracellular domains of cadherins and that this is required for ameloblasts to move in rows to form decussating enamel rod patterns.

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Toll-Like Receptor Signaling in B Cell-Mediated RANKL-Dependent Periodontitis Bone Resorption

Xiaozhe Han, Toshihisa Kawai, and Martin A. Taubman

Abstract. While various lymphocyte subsets express multiple Toll-like receptors (TLRs), the role of TLR signaling on B cell-mediated bone resorption is entirely unclear. We thereby examined the activation of TLR4 and TLR9 on RANKL production in B cells. Rowett rat splenocytes were cultured in the presence or absence of *E. coli* LPS and/or stimulatory CpG ODN. Cells were then harvested for the detection of TLR4, TLR9, and RANKL expression at both RNA and protein levels, and to determine B cell apoptosis. RANKL expressions were increased after treatment with LPS or CpG ODN alone. However, these increases were diminished when cells were treated with both LPS and CpG ODN ($p < 0.05$). Cells cultured with both LPS and CpG ODN demonstrated a significantly decreased number of RANKL-expressing B cells and an increased number of apoptotic B cells ($p < 0.01$). This study suggests that co-activation of both TLR4 and TLR9 diminishes RANKL production, probably via the induction of RANKL-expressing immune B cell apoptosis.

Key words. Apoptosis, B lymphocytes, Periodontal bone loss, Receptor activator of nuclear factor-kappa B ligand, Toll-like receptor

1 Introduction

Toll-like Receptors (TLRs) are pattern-recognition receptors (PRR) that are responsible for the initiation of innate and adaptive immune responses. They serve to identify conserved microbial components to activate immune cell responses. Lipopolysaccharide (LPS) is a bacterial endotoxin complex found on the surface of

X. Han (✉), T. Kawai, and M.A. Taubman
Department of Immunology, The Forsyth Institute,
245 First Street, Cambridge, MA 02142, USA
e-mail: xhan@forsyth.org

bacteria and is known to interact with TLR4 [1]. Unmethylated cytosine-guanosine (CpG) oligonucleotides are immunoactive motifs that are present in bacterial DNA, which are known to interact with TLR9 [2]. Stimulation of TLRs can regulate inflammatory cytokine production, modulate osteoclastogenesis through the RANKL-RANK system, and control cell survival [3].

TLRs are primarily expressed in dendritic cells (DCs) and macrophages, yet TLR expression has also been found in lymphocytes [4]. Specifically, TLR2, TLR4, and TLR8 expression has been found in CD4+CD25+ Treg cells as well as activated and memory CD8+ T cells [5, 6]. B cells from periodontal disease patients express surface Toll-like receptor 4 [7].

We hypothesize that TLR signaling plays a role in the immune B cell-mediated osteoclastogenesis. This study was to determine whether co-activation of TLR4/9 signaling regulates immune B cell RANKL production. This will help us better understand the mechanism of immune B cell-mediated periodontal bone resorption in periodontitis.

2 Results

As detected by RT-PCR, TLR4 expression was increased after treatment with LPS, and TLR9 expression was elevated after treatment with CpG ODN, but not with scrambled ODN. TLR4 and TLR9 expressions were elevated in a time- and dose-dependent manner. RANKL mRNA expressions were increased after treatment with LPS (2.5-fold) or CpG ODN (threefold) alone. However, these increases were diminished (five- and sixfold, respectively) when cells were treated with both LPS and CpG ODN ($p < 0.05$). mRANKL-expressing cells were significantly reduced when treated with both LPS and CpG ODN (5%) as compared to those treated with LPS (10%) or CpG ODN (12%) alone ($p < 0.01$). Cells cultured with both LPS and CpG ODN demonstrated a significantly decreased number of RANKL-expressing B cells and increased number of apoptotic B cells ($p < 0.01$) as compared to cells cultured with only LPS or CpG ODN.

3 Conclusion

Activation of TLR4 or TLR9 alone up-regulates immune cell RANKL expression. However, co-activation of both TLR4 and TLR9 diminishes RANKL production, probably via the induction of RANKL-expressing immune B cell apoptosis, a potential mechanism to maintain normal bone homeostasis.

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Immediate Implant Stability and Function: Biomechanics and Electron Microscopy

Hatice Hasturk, Alpdogan Kantarci, and Thomas E. Van Dyke

Abstract. Primary implant stability and lack of micromovements are considered to be two of the main factors necessary for achieving predictable high success of osseointegrated oral implants. The aim of this pilot study was to evaluate Hard Tissue Replacement-Polyanhydride-light hardened (HTR-PLH); HTR-LH: light hardened HTR; and PLH: polyanhydride synthetic bone substitute-light hardened on implant stability and function during immediate implant placement and loading in minipigs. Following immediate implant placement into fresh extraction sockets of 48 maxillary and mandibular teeth, the crestal voids surrounding the implants and infrabony spaces were treated with one of the afore-mentioned test materials or left untreated (control). At 12 weeks of loading, block sections containing implants were obtained. Evaluations included periodontal probing, pullout force load and mobility measurements to determine implant stability, radiographic evaluations to examine bone levels, and scanning electron microscopy-energy dispersed spectroscopy. The results showed that the newly formulated chemically hardened graft material, HTR-PLH, was beneficial in immediate implant placement and resulted in greater stability during immediate loading.

Key words. Alveolar ridge augmentation, Immediate dental implant, Immediate loading, Synthetic bone graft

H. Hasturk (✉), A. Kantarci, and T.E. Van Dyke
Department of Periodontology, The Forsyth Institute, 245 First Street,
Cambridge, MA 02142, USA
e-mail: hhasturk@forsyth.org

1 Introduction

Immediate implant placement has been demonstrated to be a reliable method for replacing the lost dentition [1–4]. Hard Tissue Replacement (HTR[®]) is an FDA cleared, resorbable, synthetic bone replacement used as bone void filler. HTR[®] is composed of PMMA (poly-methyl-methacrylate), PHEMA (poly-hydroxy-ethyl-methacrylate) and calcium hydroxide [5]. Studies with HTR polymer composite graft material have shown superior clinical results with regard to horizontal defect fill in human Class II furcations defects compared to open flap debridement or guided tissue regeneration (GTR) barriers alone [6–11]. A new technology where HTR and PA (polyanhydride) formulations are combined with a light/chemical hardening technology (LCH[™]) placed with a 3-phase delivery system has been proposed to improve implant stability, to promote longer and stronger host bone formation by slow resorption of synthetic material when immediate implant is placed. Poly (anhydride-*co*-imides) matrices are novel biodegradable polymeric materials with good mechanical properties designed specifically for orthopedic applications [12]. This new family of polyanhydrides is capable of yielding high strength, degradable networks after exposure to light [13]. Resorption is by surface erosion into the water phase [13].

The present work focuses on the biomechanical testing and microscopic analyses of LCH technology and a combination of HTR+[™] with polyanhydride as bone substitute when compared to light-hardened polyanhydride synthetic bone substitute or light-hardened HTR alone along with non-grafted group in a minipig model during immediate implant placement and loading.

2 Materials and Methods

A 3-month bone augmentation study was conducted using adult minipigs (18–24 months old) as described in the previous manuscript. Briefly, all premolar teeth (first, second, third and fourth) and the first molar at each quadrant were extracted under general anesthesia at baseline. At the same session, dental implants were placed followed by placement of restorative abutments over implants to provide primary contact and apply loading during the study. Crestal bone deficiencies and intrabony defects were treated with light cured (hardened) graft materials according to a randomization scheme. Follow-up and clinical evaluations were performed at 2, 6 and 12 weeks. Periotest measurements were used to determine implant stability (mobility). After digital radiographic images, soft tissue surrounding the neck of the implants was carefully removed with a Gracey curette. The block specimens were produced by cutting transverse sections (4–9 mm thick) from the central region of the bone specimens containing implants. The abutments were tightened and secured to prevent possible collapse of the implant neck during the pullout test.

Biomechanical testing was performed with a universal testing machine¹ on fresh sections to determine pullout force load strength of bone-implant interface. The abutment was grasped by the mouths of the Instron machine and positioned to apply a pure axial moments for reliability of the measurements. The implant-abutment was pulled apart from the integrated bone block and the peak loads were recorded by the load transducer. At the point of implant failure, the test was stopped immediately to prevent complete damage of the interface. The maximum force load, displacement at maximum load, load at auto break and displacement at auto break were calculated and transferred to the computer file by the Instron Software program. The full-scale load range was 10,000 kN and crosshead speed was 0.5 mm/min. In total 23 implant blocks (7 HTR-PLH; 8 PLH, 4 HTR-LH and 4 No Graft) were tested.

For scanning electron microscopy, implant-bone blocks ($n=23$) were sagittally cut using the Exakt 300 parallel band saw and gradually dehydrated. For optimum preservation of details, surfaces were left uncoated and analyzed using a Hitachi S-3000N scanning electron microscope.² Electron microscope analysis was conducted using the same Hitachi S-3000N scanning electron microscope with an Oxford Inca Energy-dispersive X-ray (EDX) system and a light element X-ray detector³ (15 mm WD). Elemental composition was determined as weight percent and calcium/phosphate (Ca/P) ratios were calculated from five measurements per area selected.

The implants were evaluated to determine the chemical composition of implant-bone interface by EDX system connected to a scanning electron microscope (SEM). The system is able to detect the atoms with an atomic weight equal to or greater to that of boron, and allows semiquantitative determination of composition of a surface within a thickness range of about 1 μm with a high resolution. Energy dispersive X-ray spectroscopy (EDS) was used to identify and evaluate the relative concentrations of all the chemical elements present in the tissues and was carried out using point analysis, line-scan and mapping facilities with EDX system. More than 200-point analyses were carried out on the sections. These included at least three sites within bone in areas with no visible adjacent biomaterial in each section, to determine the elemental composition of the natural bone as a base-line for comparison. Additional information was obtained from line scans and elemental maps.

Mean values were compared using one-way analysis of variance (ANOVA) with post hoc LSD correction. In addition, the Ca:P ratios were analyzed using ANOVA with LSD correction.

¹ Instron Model 4202, Canton, MA.

² Hitachi Instruments Engineering Co., Ibarki, Japan.

³ Oxford Instruments Analytical, High Wycombe, Bucks HP123SE, UK.

3 Results

3.1 Implant Stability

Periotest values (PTVs) recorded in the study were ranging between -7 and $+1$, which all showed a clinical stability for all implants. PTVs were recorded at 2 weeks following immediate loading and repeated at 6 and 12 weeks. The mean PTV was calculated between groups for all implants. Based on the periotest analysis, HTR-PLH displayed higher stability on average compared to all other groups throughout the study ($p=0.004$, $p=0.03$, and $p=0.04$ compared to PLH, HTR-LH, and no graft groups, respectively). Considering all implants placed either in maxilla or mandible, HTR-PLH group showed better periotest values at each time point starting at 2 weeks but the difference was not statistically significant when compared to other groups ($p>0.05$).

The mean PTV was also calculated separately for implants placed in maxilla and placed in mandible. At 2 and 12 weeks, the periotest values of implants augmented with HTR-PLH were found statistically significantly better compared to HTR-LH group when only maxillary implants were evaluated. When implants placed into mandible were considered, HTR-PLH displayed better stability at 2 weeks compared to no graft group ($p=0.05$). There was no statistically significant difference between no graft group and any of the other graft materials tested.

Mechanical pullout tests of all the implants showed similar bond strength. The mean peak values and standard deviations were calculated by the Instron mechanical analysis software and statistical analysis were performed. According to this evaluation the mean max load at breakage for HTR+PLH, PLH, HTR-LH and no graft was 0.78 ± 0.05 , 0.69 ± 0.15 , 0.71 ± 0.06 , and 0.69 ± 0.14 , respectively. Although, HTR-PLH treated bone showed slightly more strength to pullout test, statistically there was no difference between any of the groups. The displacement distance at the maximum load was also similar for all groups.

The implants were evaluated using field emission SEM. SEM microphotographs revealed well osseointegrated implant surfaces in the HTR-PLH treated sites (Fig. 1). Healthy ultrastructure and appearance of the interface was found at the places of direct contact between the implant and bone. The newly formed bone in HTR-PLH treated sites appeared to be well-organized with less marrow spaces and well-distributed osteocytes, while the peri-implant bone of the PLH and HTR-LH treated sites appeared rich in marrow spaces and less organized with poorly distributed osteocytes. The sites without augmentation showed large bone marrow spaces and poorly distributed osteocytes around bone-to implant contact area (Fig. 1).

3.2 Implant-to-Bone Contact

Scanning electron micrographs revealed a tighter implant socket interface in HTR-PLH group compared to PLH, HTR-LH and no graft groups, with reduced microfissures and dentate bone interface fractures. In detail, the SEM analysis revealed that

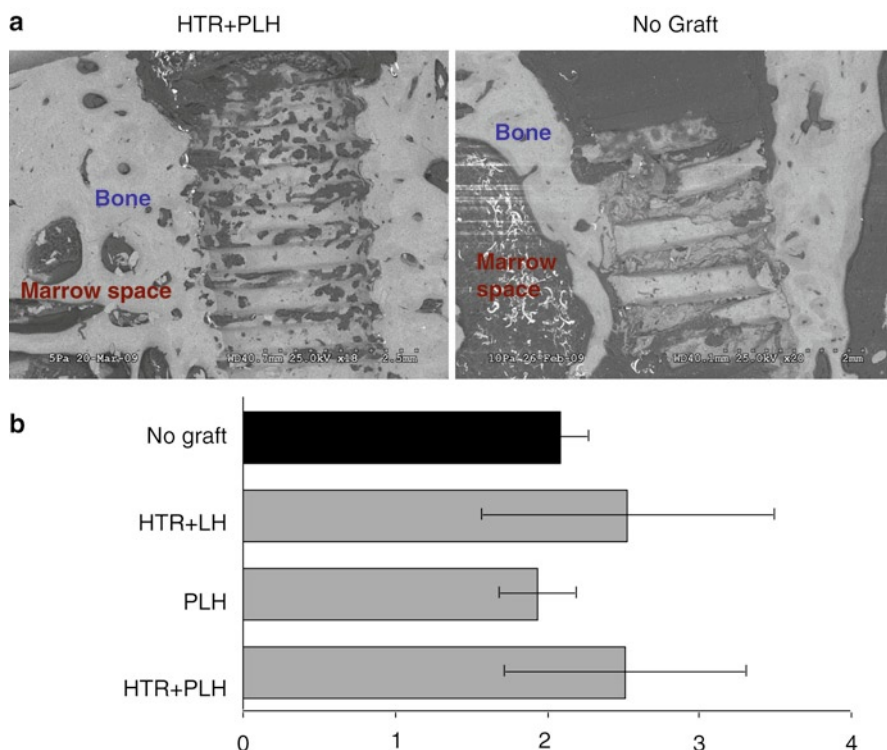


Fig. 1. Scanning electron microscopy (SEM) and energy dispersed X-ray (EDX) analysis. Implant-bone interface and the composition of the newly formed bone around implants were evaluated using field emission SEM in each group. (a) The SEM analysis revealed that sites treated with HTR-PLH showed least microfissures between implant and bone, least amount of fractures in dentate bone interface after pull-out test. Well osseointegrated implant surfaces with a healthy ultrastructure and appearance of the interface were observed. The micrographs revealed large bone marrow spaces and poorly distributed osteocytes around bone-to implant contact area in the sites without augmentation. Approximately 20 μm microfissures were detected between implant and bone and fractured dentate bone interface due to pull-out test. (b) In all groups, implant-bone surfaces were mainly composed of oxygen, calcium, phosphorus, nitrogen and carbon elements with a difference in the oxygen level in sites without augmentation. Ca/P ratios were calculated at five different areas for each sample and the mean $\pm$ standard deviation was used for statistical comparisons. Although HTR-PLH and HTR-LH showed higher Ca/P ratios compared to PLH and No graft groups, this difference did not reach to statistical significance ($p > 0.05$)

sites treated with HTR-PLH showed least microfissures between implant and bone, least amount of fractures in dentate bone interface after pull-out test. Conversely, the implants treated with PLH showed approximately 10 μm microfissures between implant and bone, “brittle” bone interface with fractures of dentate protrusions due to pull-out test. In parallel, HTR-LH group also resulted in approximately 10 μm microfissures between implant and bone with fractured dentate bone interface, however showed better integration compared to no-graft group. No graft group showed

approximately 20 μm microfissures between implant and bone and fractured dentate bone interface due to pull-out test. Energy dispersed X-ray analysis demonstrated that the surface was mainly composed of oxygen, calcium, phosphorus, nitrogen and carbon elements. EDX results showed that the surface of implants without augmentation was mainly composed of calcium and phosphorus, but little oxygen, while the HTR-PLH, and PLH treated sites were mainly composed of Calcium and phosphorus but also rich in oxygen levels (Fig. 1). The HTR-LH sites showed high oxygen levels however, the calcium and phosphorus levels were less.

4 Conclusions

The newly formulated chemically hardened graft material HTR-PLH was beneficial in immediate implant placement following tooth extraction and resulted in greater stability during immediate loading over a 3-month period. Within the limits of the small sample size, these findings demonstrate a proof-of-concept approach showing that the chemically hardened, poly-methyl-methacrylate, poly-hydroxy-ethyl-methacrylate and calcium hydroxide composite bone replacement graft material containing polyanhydride can be safely and successfully used to perform crestal ridge augmentations around implants and to provide an immediate bonding strength for the stability of implants during immediate loading.

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The Effect of Vitamin A on Bone Resorptive Lesions of Periradicular Periodontitis In Vivo and In Vitro

Jennifer Hong, Marcelo J.B. Silva, Mikihiro Kajiya,
Emad Alshwaimi, Hajime Sasaki, Peter Ok, Robert R. White,
Tom C. Pagonis, Bruce J. Paster, Philip Stashenko,
and Toshihisa Kawai

Abstract. Using a mouse model of periradicular periodontitis, we examined the effect of vitamin A (Vit A) deficiency on the osteoclastogenesis in the periadicular lesion. In addition, we explored the suppressive effect of Vit A on the RANKL production from endodontic bacterium-activated T cells in vitro. After 14 days of artificial pulp exposure, TRAP-positive osteoclast-like cells were dramatically observed in periapical region. Most importantly, the numbers of TRAP-positive osteoclast-like cells were significantly decreased in the Vit A-deficient group in comparison to control group (Student's *t*-test, $P < 0.05$). Furthermore, in vitro study showed that Vit A treatment abrogated the production of RANKL in T cells activated by *Pasteurella pneumotropica* which is identified in mouse periapical lesion by using 16s-ribosomal RNA technique. These findings suggested that the dietary Vit A appeared to down-regulate the pathological bone resorption, possibly by its immune-suppressive effect.

Key words. Bone resorption, Osteoclast, Periradicular periodontitis, RANKL, T cells, Vitamin A

J. Hong, M. Kajiya, E. Alshwaimi, H. Sasaki, P. Ok, B.J. Paster,
P. Stashenko, and T. Kawai (✉)
Harvard School of Dental Medicine, Boston, MA, USA
and
The Forsyth Institute, Boston, MA, USA
e-mail: TKawai@forsyth.org

M.J.B. Silva
The Forsyth Institute, Boston, MA, USA

R.R. White and T. C. Pagonis
Harvard School of Dental Medicine, Boston, MA, USA

1 Introduction

The pathogenesis of apical periodontitis derives mostly from endodontic bacterial infection, which results in bone resorption and destruction of connective tissue around the root apex [1–3]. The currently applied therapeutic approach involves the chemo-mechanical removal of etiologic components present in the affected root canal, or so-called root canal therapy. Although root canal therapy has a success rate of 96% in necrotic teeth without apical periodontitis, the success rate drops to 86% when apical periodontitis is involved [4]. The cases are deemed successful when the bone resorption lesion disappears within one year after appropriate root canal treatment [5]. However, when root canal treatment fails to ameliorate the bone resorption lesion, either the affected root apex is surgically removed by apicoectomy or the whole affected tooth is extracted. In the context of periradicular periodontitis, it is implicated that receptor activator of nuclear factor κ B-ligand (RANKL) produced from activated T cells may be associated with the development of pathogenic bone resorption lesion [6]. On the other hand, while vitamin A is known to have a regulatory effect on adaptive immune responses [7, 8], its influence on RANKL production from T cells and resulting periradicular bone resorption remains unclear. Therefore, in this present study, we observed the effect of Vit A on the bone resorptive lesion of periradicular periodontitis in vivo and in vitro.

2 Materials and Methods

Mice were fed a normal diet with sufficient vitamin A (Vit A) (control) or a vitamin A-depleted diet for 28 days and respectively received pulp exposure in mandibular first molars ($n=5$, each group). Subsequently, exposed pulps were left open to the oral environment for 0 and 14 days. Osteoclastogenesis occurring in the periradicular bone was assessed by in situ tartrate-resistant acid phosphatase (TRAP) staining. In order to identify the endodontic bacterium that can activate T cells to produce RANKL, 16S-ribosomal RNA-based bacteria identification was employed. The identified bacteria from exposed pulp were cultured and used as T cell antigen in the in vitro T cell assay. The effect of Vit A on RANKL expression induced by in vitro-activated T cells in response to their specific antigen was monitored using ELISA.

3 Results and Discussion

In the control group of mice, TRAP (+) osteoclasts were identified in the radicular tissue surrounding the pulp-exposed tooth and the number of TRAP (+) osteoclasts in the periradicular lesion decreased in the group of mice fed with a Vit A-deficient diet. *Pasteurella pneumotropica* (Pp) was found from the exposed pulp as an

immuno-dominant bacterium that induces sRANKL producing T cells. Most importantly, Vit A treatment abrogated the production of RANKL in T cells induced by in vitro activation with *Pp*.

It is conceivable that an approach that can suppress RANKL expression by activated T cells may lead to the amelioration of pathogenic bone resorption in the infectious lesion of apical periodontitis. In this regard, dietary Vit A showed remarkable suppressive effects on the production of RANKL induced by *Pp*-reactive T cells in vitro. Vit A is attracting interest in research involving Treg cells because it can also increase the immune-suppression activity of Treg cells [9, 10]. Indeed, Vit A depletion in the diet reduced the FOXP3+ Treg cells in the cervical lymph nodes of mice compared to the cervical lymph nodes of mice that received regular diet (unpublished data), suggesting that Treg cells may be involved in the Vit A-mediated down-regulation of T cell-RANKL-induced periradicular bone resorption. While additional studies are required to evaluate if Treg cells are involved in the Vit A-associated suppression of bone loss in the periradicular periodontitis, the immunomodulatory effects of Vit A that suppressed the RANKL expression from activated T cells may lead to the development of a novel therapeutic regimen for periradicular periodontitis.

4 Conclusion

Pulp exposure in mice elicits the infiltration of RANKL-positive T cells in periradicular lesion in conjunction with the induction of local osteoclastogenesis, but dietary Vit A appeared to down-regulate pathological bone resorption, possibly by its immune-suppressive effect.

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Immediate Implant Stability and Function: A Minipig Model and Surgical Technique

Hatice Hasturk, Alpdogan Kantarci, and Thomas E. Van Dyke

Abstract. A successful osseointegrated oral implant is anchored directly to bone, however, in the presence of movement; a soft tissue interface may encapsulate the implant causing its failure. Therefore, immediate implant placement and immediate loading where a primary stability is limited have been considered harmful for a successful osseointegration. The present study was designed as a proof of concept study to evaluate a new technology where the light/chemical hardening could enhance the ability of bone substitute material to build an immediate and stable connection with the implant surface at the crestal level and would both increase the primary stability and soft tissue support around the neck of the implant. To test this hypothesis, a newly formulated poly-methyl-methacrylate, poly-hydroxyl-ethyl-methacrylate, and calcium hydroxide plus polyanhydride composite graft material as bone substitute was compared to positive and negative controls in a minipig model. The results of the study demonstrated that the new combination of bone substitute provides better soft tissue and radiographic bone level around immediate implants.

Key words. Alveolar ridge augmentation, Animal model, Immediate implant placement, Immediate loading

1 Introduction

Primary implant stability is considered the main factor for achieving success of osseointegrated oral implants [1]. A key to predictability of the titanium implant is the adequate remodeling of peri-implant bone where bone-to-implant contact at the apical or coronal level at placement represents the top priority for the stability and

H. Hasturk (✉), A. Kantarci, and T.E. Van Dyke
Department of Periodontology, The Forsyth Institute,
245 First Street, Cambridge, MA 02142, USA
e-mail: hhasturk@forsyth.org

prognosis of the implant [2, 3]. The bone-to-implant contact rate is predictable when anatomy allows a close fit insertion of the implant [4]. Until now, the peri-implant bone density and the bone mineral apposition rate have not been quantified for immediately loaded implants.

Immediate implant placement has been demonstrated to be as reliable as traditional surgical techniques [5–8] while problems relate to primary stability and bone healing on implant surfaces at the coronal level [2, 3, 9]. In order to overcome gaps between implant surfaces and bone, immediate implant placement is recommended by guided bone regeneration with barrier membranes and/or bone grafts [10, 11]. On the other hand, several studies have shown that healing and osseointegration rate did not differ when barriers were used [12–14]. This issue is critical for success and many novel materials have been continuously introduced to the market with only limited studies validating the claims by the producers.

The present pilot study is designed as a proof of concept study to evaluate a new formulation of a bone substitute in a minipig model on implant stability and function during immediate implant placement and loading. This first part presents the animal model, surgical technique and clinical findings.

2 Materials and Methods

The technology and products used in this study were HTR-PLH (new combination bone substitute; light hardened HTR and polyanhydride combination); HTR-LH: light hardened HTR; PLH: light hardened polyanhydride synthetic bone substitute, and original HTR (hard tissue replacement graft material). These products have been obtained from Bioplant R&D, LLC (Westport, CT). HTR-PLH is a biocompatible micro-porous composite of PMMA (poly-methyl-methacrylate), PHEMA (poly-hydroxy-ethyl-methacrylate) and calcium hydroxide bone replacement graft material containing polyanhydride. HTR-PLH composite graft material and PLH were applied manually using a 3-phase system and hardened *in situ* with standard dental curing light to support immediate implants placed in fresh extraction sockets in minipigs.

Study protocol and experimental design were reviewed and approved by the Boston University Medical Center Institutional Animal Care and Use Committee (BUMC IACUC) prior to study initiation. Three Göttingen® minipigs (adult male; 18–24 months old; average weight 35 kg) were used. The minipigs were housed separately and fed with regular minipig diet and tap water *ad libitum*. Following the implant surgeries, the animals were placed on a semi-liquid diet for 2 weeks. All surgical and post operative oral hygiene care procedures were performed in aseptic conditions and under general anesthesia. Local anesthesia with 2% Lidocaine with 1:100,000 epinephrine was administered to the surgical sites to obtain local vasoconstriction and to prevent discomfort during extractions and implant placement. The animals were anesthetized at 2, 6 and 12 weeks for postoperative oral care, clinical assessments and implant stability tests.

Maxillary and mandibular premolar (four premolars per quadrant) and molar teeth (one molar tooth per quadrant) were extracted. Immediately after extractions, the sockets were irrigated with saline solution to remove any remnant of the teeth/roots separated. Sockets (mesial root of each premolar tooth) were prepared and implants (Parallel wall, screw-type, 3.25 mm in diameter and 11.5 and 13 mm in length titanium implants¹) were placed. The insertion torque was 40 Ncm for each implant. Where necessary, the placement was completed with hand ratchet to optimally position the implant platform in order to maximize primary stability. The implants were inserted until the coronal aspect was completely under the bone. A total of 48 implants, 16 implants in each pig, were inserted. The healing abutments (4 mm collar height)² were tightly screwed into the implants (internal connection) 2–3 mm away from the opposing occlusion for controlled immediate loading and left unsplinted.

The crestal areas and all bony sites at the boundaries of implants were filled with either HTR-PLH or HTR-LH or PLH graft material using 3-phase delivery system and dental curing light was used to harden the graft material for implant augmentation according to manufacturer's guidelines. The implant sites assigned to receive no graft (control) were left untreated. The placement of the graft material around the implants and on the crestal areas constituted a modified ridge augmentation procedure insuring tight closure without any exposure of the surface and flaps were primarily closed using 4–0 resorbable sutures.

Antibiotic (Ceftiofur Sodium SID, 3–5 mg/kg, IM)³ and analgesic therapies (buprenorphine peri-operatively at 0.02–0.05 mg/kg IV q4–6 hours, then IM BID for 2–3 days post-operatively; ketoprofen 3 mg/kg IM intra-operatively and meloxicam 0.4 mg/kg p.o SID x 3–4 days post-operatively) were administered. Animals were checked daily for the first week after surgery to confirm safety. All animals were anesthetized using general anesthesia for a clinical follow-up and oral hygiene including brushing and irrigation with chlorhexidine digluconate at 2 and 6 weeks post-operatively.

At the end of the 12 weeks, animals were anesthetized with ketamine HCL/xylazine IM and euthanized by pentobarbital overdose (120+mg/kg, iv). Maxillas and mandibles were removed en bloc using oscillating bone saw and high speed cutting discs under continuous saline irrigation and two bone blocks were obtained. Each block containing four dental implants was further cut with a diamond saw under continuous cooling water irrigation and each implant was separated with the surrounding bone. Half of the block sections ($n=23$; one implant was lost at 6 weeks postoperatively) containing implants were used for clinical and mechanical analysis and then for undecalcified sectioning for further analysis with scanning electron microscopy-energy dispersed X-ray spectroscopy (SEM-EDS).

¹ Osseotite® Certain® Microminipiant™, Biomet 3i, Inc.

² Biomet 3i, Inc.

³ Naxcel®, Pfizer Inc, 235 E. 42nd St. New York, NY USA.

Clinical and macroscopic evaluations included visual inspection, periodontal probing of the peri-implant sulcus, application of force (pullout force load) and Periotest measurements to determine implant stability (mobility) and radiographic evaluations to examine radiographic bone levels. A UNC15 periodontal probe was used to measure the probing attachment level at six point of each implant at 12 weeks immediately after removal of the jaws. The same examiner performed all probing measurement to avoid intraexaminer variability. Radiological bone loss around implants was evaluated by counting the implant threads outside of bone at both sides (mesial and distal). The data obtained by direct measurements during morphologic assessment were used in multiple statistical analyses. Mean values were compared using one-way analysis of variance (ANOVA) with post hoc LSD correction.

3 Results

All surgical sites healed without major complications. A few sites exhibited some minor gingival inflammation. At 6 weeks, one implant was lost most probably due to trauma. Occlusal surfaces of the abutments exhibited contact points (tattered and shining), which indicated function. At sacrifice, all implants showed good healing results and clinical stability except the one lost at 6 weeks.

Figure 1 shows the clinical soft tissue and radiographical bone examinations. The probing pocket depths around implants did not indicate any pathological pocket formation or bone loss. HTR-PLH was slightly better than the other groups but there was no statistically significant difference between groups with respect to the pocket depth ($p > 0.05$). The bone loss around implants was calculated by counting the exposed implant threads on the digital radiographs as well as any radiolucency between the implant-bone interfaces. Immediate implant placement and immediate loading resulted in bone at or slightly apical to the first thread of the implant in all groups at 12 weeks. Radiographs did not reveal any significant radiolucency at the crestal or apical bone or at the implant-bone interface along the implant. The exposed thread numbers at the crestal level varied between 0 and 3, and PLH group demonstrated the highest number of threads exposed among the groups (mean $\pm$ SD = 2.6 ± 0.8 ; $p < 0.05$), while the implants augmented with HTR-PLH had thread exposure at very minimal level (0.8 ± 0.9).

4 Conclusions

This study demonstrated that the minipig model is a suitable in vivo system where immediate implantation and efficacy of the bone substitutes could be studied. In addition, the results of the study demonstrated that the new combination of bone substitute provides better soft tissue and radiographic bone level

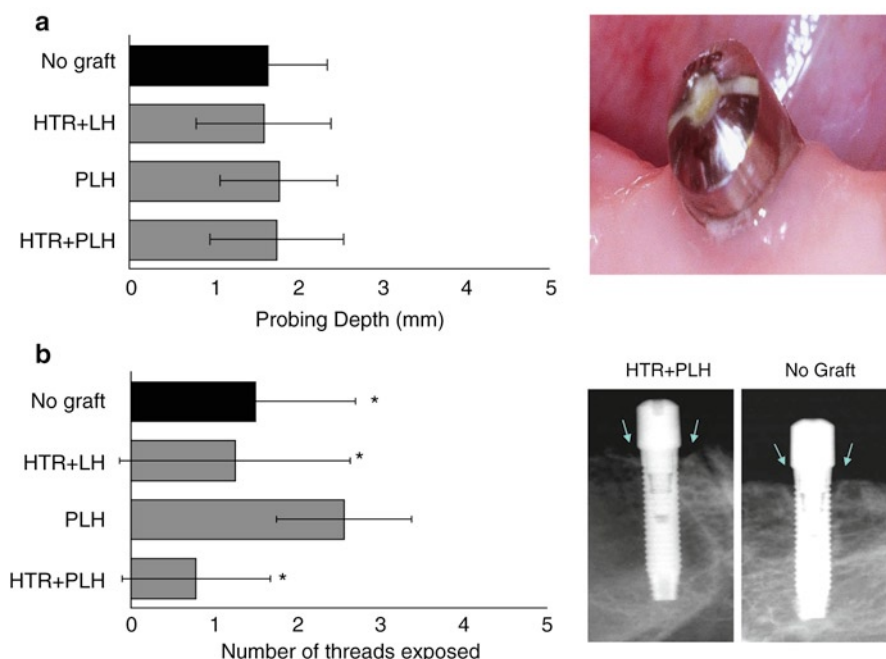


Fig. 1. Soft tissue attachment and radiographic bone level around implants. **(a)** Soft tissue probing was made at six points around each implant. Mean $\pm$ SD was compared between groups. No statistically significant difference was found between groups ($p > 0.05$). **(b)** Digital radiographs were obtained after sample harvesting (ex-vivo) using Schick technology. The radiographs were evaluated for counting implant threads exposed and any radiolucency between the implant-bone interfaces using the software. PLH group demonstrated the highest number of threads exposed among the groups (mean $\pm$ SD = 2.6 ± 0.8 ; $*p < 0.05$), while the implants augmented with HTR-PLH had minimal tread exposure (0.8 ± 0.9 ; $*p < 0.05$ compared to PLH group)

around immediate implants. The periodic assessments indicate that HTR-PLH can facilitate a stable implant-bone interface which in turn can increase the survival rate of immediate implants where immediate loading is desirable.

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Detection of RANKL and OPG in Chronic Periradicular Periodontitis

Peter Ok, Jun-O Jin, Mikihiro Kajiya, Christine Min, Kazuhisa Ouhara, Jennifer Hong, Robert R. White, Tom C. Pagonis, Philip Stashenko, and Toshihisa Kawai

Abstract. This study examined the cellular sources of RANKL in mononuclear cells infiltrating the lesion of periradicular periodontitis, including B and T lymphocytes and monocytes. Also, mRNA expression of RANKL and OPG was compared between periradicular tissues of patients with chronic periradicular periodontitis and normal healthy periradicular tissues sampled from extracted wisdom teeth. Immunofluorescent confocal microscopy analysis shows that T and B lymphocytes, as well as monocytes, express RANKL. Results from quantitative RT-PCR indicated that the expression ratio of RANKL mRNA over that of OPG mRNA was a more critical determinant of disease pathogenesis than the titers of either RANKL mRNA or OPG mRNA detected in the sampled tissues, strongly suggesting that the balance between RANKL and OPG is pertinent in periradicular bone pathology. In conclusion, RANKL is expressed by lymphocytes as well as monocytes and the balance of RANKL/OPG expression could also be a determinant of pathogenic bone resorption in chronic periradicular periodontitis.

Key words. Chronic Periradicular Periodontitis, Immunofluorescent confocal microscopy, mRNA, OPG, RANKL

P. Ok, M. Kajiya, J. Hong, P. Stashenko, and T. Kawai (✉)
Harvard School of Dental Medicine, Boston, MA, USA
and
The Forsyth Institute, Boston, MA, USA
e-mail: TKawai@forsyth.org

J.-O. Jin, C. Min, and K. Ouhara
The Forsyth Institute, Boston, MA, USA

R.R. White and T. C. Pagonis
Harvard School of Dental Medicine, Boston, MA, USA

1 Introduction

The main purpose of endodontic treatment is to maintain healthy periradicular tissues and prevent pathologic periradicular bone resorption. Periradicular lesions of endodontic origin are known to be generated by the immune system, but the exact pathobiological mechanism underlying periradicular bone resorption is not yet fully understood. Both receptor activator of NK-kappa B ligand (RANKL) and Osteoprotegerin (OPG) are known to be important cytokines that may be responsible for pathologic periradicular bone resorption. RANKL produced from activated T and B cells plays a role in the induction of local osteoclastogenesis in the inflammatory bone loss lesions, such as rheumatoid arthritis and chronic marginal periodontal disease [1, 2]. While previous studies using immunohistochemical staining suggested that monocytes express RANKL in the human periradicular lesion [3], it remains unclear whether lymphocytes, including T and B cells, also produce RANKL in the periradicular lesion. This study evaluated RANKL and OPG mRNA expression patterns in chronic periradicular periodontitis. In addition, the cellular sources of RANKL in the periradicular lesion were studied using immunofluorescent confocal microscopy.

2 Materials and Methods

The human tissue samples were collected during extraction or periadicular endodontic surgery at the Harvard Dental Center after approval of the Harvard Committee on Human Studies. Chronic periradicular tissue samples were collected following endodontic periradicular surgery. Healthy periradicular tissues were collected during dental extraction procedures. To detect the cellular sources of RANKL production on the mononuclear cells (CD4, CD8, CD11c, CD14 and CD19) in the periadicular tissue, we employed the immunofluorescent confocal microscopy. Using quantitative RT-PCR (qRT-PCR), RANKL and OPG mRNA expressions in the sampled periradicular tissue were compared between the healthy and periradicular periodontitis groups.

3 Results and Discussion

Confocal microscope data show that B lymphocytes (CD19) and T lymphocytes (CD4), as well as monocytes (CD14) express RANKL in periradicular tissue, suggesting that immune cells may play a key role for pathogenic bone resorption. The qRT-PCR data showed that the quantity of either RANKL mRNA or OPG mRNA does not seem to correlate with the degree of periradicular periodontitis pathogenesis observed in the healthy group compared to the chronic periradicular periodontitis

group. More specifically, the wide range of expression levels found for RANKL and OPG mRNA in the patient group did not show a significant difference from the healthy group. On the other hand, interestingly, data also showed that the ratio of RANKL mRNA expression over that of OPG mRNA was significantly different between the two groups, and that this ratio was associated with clinical chronic periradicular periodontitis.

While we know that expressions of RANKL and OPG are involved in homeostatic bone remodeling [4], it is clear, based on this study, that the balance of RANKL/OPG expression could also be a determinant of pathogenic bone resorption. It is noteworthy that RANKL production from osteoblasts and bone marrow stromal cells is engaged in homeostatic bone remodeling. Thus, a therapeutic approach that suppresses pathogenic bone resorption, but not homeostatic bone resorption, would be an ideal treatment regimen for periradicular bone loss. Specifically, the phenomenon of RANKL expression on immune cells indicates that RANKL-expressing immune cells can be an ideal target for the development of a therapeutic regimen for periradicular bone loss.

4 Conclusion

In sum, this study demonstrated that RANKL is expressed by T and B lymphocytes as well as monocytes in periapical lesion, and that the balance of RANKL/OPG expression could also be a determinant of pathogenic bone resorption in chronic periradicular periodontitis.

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Role of N-Cadherin in Intercellular Adhesion, Tissue Development, Cytoskeleton Formation, and Signaling

Megan L. Sierant and John D. Bartlett

Abstract. N-cadherin is a classical member of the cadherin super-family. Classical cadherins are transmembrane glycoproteins that mediate calcium-dependent homophilic protein–protein interaction for cell–cell adhesion and signaling. N-cadherin is a component of the adherens junction and mice with N-cadherin ablated from their genome die during early gestation. N-cadherin is also involved in several signaling pathways that may regulate cell differentiation and motility. Therefore, N-cadherin is critical for normal development and it may play a role in enamel formation because the ameloblast cells responsible for enamel formation express N-cadherin in a developmentally defined manner.

Key words. Ameloblast, Cell migration, Cell signaling, Enamel development, N-cadherin

1 Introduction

Cadherins are transmembrane glycoproteins that mediate calcium dependent cell–cell adhesion. The cadherin superfamily can be divided into classical cadherins, desmosomal cadherins, protocadherins, seven pass transmembrane cadherins, and FAT-like cadherins [1]. Classical cadherin family members are the transmembrane components of adherens junctions (AJ). E-cadherin (epithelial cadherin), N-cadherin (neuronal cadherin), and P-cadherin (placental cadherin) are classical cadherins.

M.L. Sierant and J.D. Bartlett (✉)

Department of Cytokine Biology, The Forsyth Institute, 245 First Street,
Cambridge, MA 02142, USA
and

Department of Developmental Biology, Harvard School of Dental Medicine,
Boston, MA 02115, USA
e-mail: jbartlett@forsyth.org

Although their names imply tissue specificity, the expression of these cadherins is not completely tissue-specific and cadherin function is critical in normal development [2].

Interest in cadherins increased in the late 1980s when they were found to be associated with development and when their dysfunctional regulation was found to be associated with cancer and other disease. Cadherins form cell–cell adhesions by homophilic protein–protein interaction of extracellular cadherin domains among neighboring cells. This occurs in a zipper-like fashion and structural linkage of the cadherin intracellular domain to the cell cytoskeleton is required for full functionality. The strength of cadherin interaction can be regulated by a number of proteins, including the catenins, which serve to link the cadherin to the cytoskeleton. Cadherins have been implicated in a number of signaling pathways that regulate cellular behavior and it is becoming increasingly clear that integration of information received from cell–cell signaling, cell–matrix signaling, and growth factor signaling plays a role in cellular phenotype and behavior [3].

To make a cell motile, various factors work in tandem as motility and adhesion must be tightly regulated so that only the desired cells become migratory. The assembly of the AJ complex is highly regulated. It begins with binding of β -catenin to the carboxyl terminus of a cadherin in the presence of p120 catenin and this complex moves to the plasma membrane where it recruits α -catenin which, in turn, binds with actin filaments. An individual actin–cadherin unit can be clustered and cross linked with adjacent cadherins and actin filaments making a strong focal point. p120 promotes lateral clustering of cadherin molecules necessary for strong cell adhesion. Cadherins are required for the maintenance of tissue integrity. Loss of E-cadherin has been associated with changes of cell morphology, motility, and poor prognosis of various cancers. E-cadherin is well known repressor of metastasis [4]. A change in expression from one cadherin to another within a tissue is termed cadherin switching and is associated with initiation of cell motility and/or defining a different tissue layer [5].

2 N-Cadherin Nomenclature

N-cadherin was discovered in neural cells. It was identified as a 130 kDa protein in the chicken neural retina that is protected by calcium from proteolysis [6]. It is also known as Neural cadherin or Cadherin-2 and was identified originally as the cell adhesion molecule A-CAM [7]. The protein has also been referred to also as CDHN [neural calcium-dependent adhesion protein].

3 N-Cadherins in Development

Cell adhesion molecules instruct cells to remain in a particular site, to associate specifically with neighboring cells or to disrupt their associations and migrate directionally. These molecules have key roles in animal morphogenesis. The differential

expression of cadherins determines tissue segregation and cell sorting during development. The experimental sorting of dissociated embryonic cells back into their respective tissues was demonstrated almost 50 years ago (reviewed in [8]). Since that time, it has become clear that cadherins constitute a major cellular adhesion/recognition system. It has even been shown that cells expressing differing levels of the same cadherin will become sorted into their respective expression level groups [9]. Therefore, when a group of cells separates from an existing cell layer, the segregating cells are expressing a qualitatively or quantitatively altered set of cadherin family members. N-cadherin is required during gastrulation for proper establishment of the left–right axis [10]. N-cadherin is essential for the proper development of the yolk sac and myocardium in mouse embryos. Knock-out mice lacking expression of N-cadherin die early during the gestation period. These embryos display major heart defects and malformed neural tubes and somites. The defect can be rescued partially by re-expression of N-cadherin [11]. The N-cadherin chimeric mouse, having both N-cadherin positive and negative cells, survived long enough for the interactions between N-cadherin negative and N-cadherin positive cells to be examined. Interestingly, N-cadherin-deficient cells sorted away from N-cadherin positive cells in the brain and heart. This demonstrated that absence of a single cadherin family member mediated sorting of otherwise identical cells within a developing embryo [12].

It has been reported that N-cadherin is involved in the formation of central nervous system synapses [13]. In addition, its expression in early CD34(+) CD19(+) hematopoietic cells points to its involvement in the development and retention of early hematopoietic cells in the bone marrow [14]. N-cadherin also plays a role in the commitment, condensation and chondrogenic differentiation of mesenchymal cells during osteogenic and myogenic differentiation [15].

4 N-Cadherin and Epithelial to Mesenchymal Transition

Epithelial to mesenchymal transition, in which epithelial cells are converted to motile, fibroblast-like cells, are regular events during normal embryonic development. Similar events are seen as epithelial cells progress through the stages of carcinogenesis. This involves cadherin class switching (CS). In the context of tumour biology CS represents an important aspect of the epithelial to mesenchymal transition (EMT), a process by which epithelial cells lose their characteristic polarity, disassemble their cell junctions, become more motile and act as precursors for tissue invasion or metastasis. This switching may also change intracellular signaling to enhance growth and tissue invasion. In a malignant setting, the CS is typically from E-cadherin to N-cadherin [16]. The mechanism behind the CS and the associated change in cell signaling is not well understood. Matrix metalloproteinases (MMPs) degrade extra cellular matrix proteins and it is established that during initiation of various carcinomas (e.g., breast, prostate, bladder, thyroid) their secretion increases as does expression of N-cadherin [17].

5 N-Cadherin in Tooth Development

Enamel development progresses through defined stages marked by changes in morphology of the ameloblasts of the enamel organ. Ameloblasts are a single layer of tall, columnar cells that are responsible for enamel development at their apical cell surface and attach to the stratum intermedium of the enamel organ at their basal end. Secretory stage ameloblasts secrete proteins into the enamel matrix as the soft enamel grows to its full thickness. This is when the ameloblasts move in rows to form the characteristic enamel rod pattern. The ameloblast then transition (transition stage) into shorter maturation stage cells that reabsorb the proteins from the enamel matrix. This is when enamel achieves its final hardened form. Once the enamel is fully mature, ameloblasts regress and become part of the reduced enamel organ that covers and protects the completed enamel surface until the tooth erupts. Therefore, ameloblasts progress through defined developmental stages that require intercellular adhesion/contact and communication. It has been shown that E-cadherin, p120-catenin and β -catenin are expressed during enamel development. Additionally, adherens junctions were previously identified along the possible sliding interface of adjacent secretory stage ameloblast rows [18]. Therefore, adherens junctions may be important for enamel formation [19].

In a study on human tooth development weak expression of N-cadherin was observed in the developing enamel organ at the sixteenth week of gestation. At this stage the enamel organ is in the cap stage and forms three distinct tissue layers: the inner enamel epithelium, the outer enamel epithelium, and the stellate reticulum. From the eighteenth to twenty-first gestational weeks, increasing growth of the enamel organ gives rise to the early bell-stage. Weak N-cadherin expression was detected in proliferating inner enamel epithelium cells and stronger staining was observed as these cells progressed to become pre-ameloblasts. From the thirtieth day onward, the tooth germs are in the late bell stage of development and as E-cadherin expression diminished, the immunoreactivity for N-cadherin in the dental epithelium increased. N-cadherin labeling became strong in secretory stage ameloblasts [20]. N-cadherin may therefore be required for ameloblasts to move in rows during the secretory stage of enamel development.

6 N Cadherin in Cell Signaling

Cells obtain information from their environment to modulate their behavior during normal embryonic development and during abnormal processes such as tumorigenesis. Such signals can be initiated by growth factors, cytokines, and other soluble signaling molecules found in the circulation and in the interstitial compartment. Alternatively, signaling cascades can be activated through interactions of cells with the surrounding extracellular matrix or with one another. Sometimes, an interaction with matrix components or with other cells stimulates the modulation of,

or promotes cross talk with, well-established signaling pathways. AJ are not static but are dynamic and perturbations in the rate of addition or subtraction of components could have significant effects on whether junctions persist, grow, or disappear.

The wnt pathway is the best studied pathway of the AJ. β -catenin can translocate from the AJ to the nucleus and bind with the TCF/LEF family of transcription factors. It can act as an activator of the wnt pathway to make cells motile. N-cadherin directly interacts with FGF receptors. Interactions with FGF receptors are required for the contact-dependent survival of ovarian granulosa cells [21]. FGF receptors also participate in regulating N-cadherin mediated cell motility and metastasis in cancer cells. N-cadherin and FGF receptor interactions have also been shown to be involved in the control of axonal growth, guidance to synapse formation, and synaptic plasticity [22]. Full length and soluble N-cadherin was shown to promote cell-matrix adhesion and neurite outgrowth [23]. Similarly, recent studies have begun to tease apart the connection between cadherin function and Rho-family GTPases, MAP kinases and receptor tyrosine kinases [3, 24].

7 Conclusions

N-cadherin is an important member of the cadherin super-family. It plays an important role in normal development and an imbalance in its expression may lead to pathological conditions. It is becoming increasingly clear that cellular context plays a critical role in determining the consequences of N-cadherin expression. For example, N-cadherin promotes strong cell–cell adhesion in cardiac muscle as a component of the intercalated disk [11, 25]. However, when tumor cells of epithelial origin express N-cadherin, it promotes decreased adhesion and increased motility and invasion. Investigations into signaling pathways downstream of cadherins are in their early stages and a better understanding of this field is needed. It will be interesting to determine if cadherins help direct ameloblast cell movement necessary to form the decussating enamel rod pattern found in rodent enamel. As we learn more about the pathways downstream of specific cadherins, we will begin to better understand the differences between cadherins and how their expression can confer diverse cellular phenotypes.

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Osteopontin in the Response to Endodontic Infection

Susan R. Rittling

Abstract. Osteopontin is a secreted phosphorylated protein with pleiotropic functions in cancer and inflammation. In a mouse model of endodontic infection, the protective effect of OPN is similar in magnitude to that of IL-10, and appears to involve the innate immune response. In the absence of OPN, there is an alteration in the neutrophil/macrophage ratio in infected tissues, suggesting that OPN regulates the accumulation of innate immune cells. This effect of OPN could be through the regulation of migration of both neutrophils and macrophages and/or through regulating some aspects of macrophage function.

Key words. Osteopontin, Infection, Integrin, Macrophage

Endodontic infection, also known as periapical periodontitis, is an infection of the dental pulp and tissue adjacent to the tooth root apex [1, 2]. These infections result from bacterial invasion through cracks in the tooth, untreated caries, or failed caries restoration. They are polymicrobial, involving a subset of oral pathogens [3–5] and result in inflammation and ultimately in destruction of the surrounding tissue including soft tissue, bone and the tooth itself. These infections are remarkably prevalent, with 50% of all Americans experiencing the disease by the age of 50 [6]. Endodontic infections are polymicrobial and are representative of numerous infections of this type, including marginal periodontitis [7], skin and soft tissue infections [8] such as foot infections common in diabetic patients [9], and infection occurring in neutropenic cancer patients [10].

While both non-specific innate immune and antigen-specific acquired responses occur in periapical and periodontal regions in response to oral infection [11–13], the first line of host defense is innate immunity. Neutrophils release various inflamma-

S.R. Rittling (✉)

The Forsyth Institute, 245 First St, Cambridge, MA 02142, USA

e-mail: srittling@forsyth.org

tory mediators such as superoxide, myeloperoxidase and IL-1 during their death after phagocytosis of pathogens [14]. Hyperfunction of neutrophils enhances tissue destruction as seen in localized aggressive periodontitis [15]. On the other hand, dysfunctions of neutrophils are well known to increase susceptibility to infection [16]. Macrophages function to remove both bacteria and apoptotic neutrophils, and interface with the adaptive immune system through cytokine production and antigen presentation [17]. The resultant T cell-mediated adaptive immune response, including IL-12-mediated Th1 responses, are involved in a pathogenic pathway of dentoalveolar infection [18], however immune suppressive IL-10 and IL-6 appear to balance the Th1 response, so that endodontic infections are primarily innate immune response dependent [18–21].

Osteopontin [OPN] is a secreted integrin-binding protein that is matrix associated, but soluble in many soft tissues [22]. The protein is present in bodily fluids, including serum, urine, CSF and milk [23]. It is secreted by a variety of cell types, and its expression is often upregulated after injury or infection [24]. The functional domains of the protein include a hydroxyapatite binding sequence that facilitates the binding of the protein to bone and a central integrin binding region that has at least two distinct integrin binding sites [25]. OPN has a random, disordered structure with little secondary structure [26]. Osteopontin maps to a locus coding for resistance to *Rickettsia tsutsugamushi*, and mouse strains carrying the allele conferring susceptibility have delayed upregulation of OPN in response to infection [27]. OPN-deficient mice are more susceptible to both *Listeria monocytogenes* and *Plasmodium chabaudi chabaudi* [28, 29]. OPN function in the innate immune system is illustrated by inefficient clearance of *Mycobacterium bovis* bacillus Calmette–Guérin in OPN-deficient mice associated with alterations in macrophage killing [30], and an initial defect in neutrophil accumulation after *Klebsiella pneumoniae* infection in OPN-deficient mice resulting in increased bacterial load and increased morbidity and mortality [31]. These data support the idea that osteopontin is required for efficient innate immune function, through enhancing either neutrophil or macrophage responses, or both.

Mouse models of endodontic infection replicate the human disease [19, 20, 32, 33]. In these models, the dental pulp of the molars is surgically exposed, as in human endodontic treatment, to reveal the root canal entrances. A mixture of common human endodontic pathogens is introduced to the pulp chamber to initiate infection, which results in a lesion forming at the apex of the molar root (periapical lesion). This model has been used to define the role of myeloid cells in these infections. Mice deficient for P/E selectins, endothelial cell molecules that are required for neutrophil rolling on and adhesion to the endothelium, have impaired neutrophil infiltration into sites of infection [34] and much more extensive periapical lesions [33]. The importance of macrophage infiltration in these infections was demonstrated in studies of MCP-1 knockout mice, where periapical inflammation was increased, in parallel with reduced macrophage, but not neutrophil, infiltration [35]; similar results were reported in mice deficient for the receptor for MCP-1, CCR2 [36]. This model has also been used to demonstrate the importance of several cytokines in these infections, especially IL-10 [19].

This model [19] was also used to examine the role of endogenous OPN in endodontic infection [37]. Three weeks after pulp exposure and infection, there was significantly increased tissue destruction and cellular infiltration in OPN deficient mice as compared to WT, accompanied by increased bone loss. The magnitude of this effect was quite large, and was similar to that seen in the hyperinflammatory IL-10-deficient mice [19], suggesting that osteopontin loss plays a major role in the development of hyper-inflammation. Although OPN has been implicated in regulation of the adaptive immune response [38], there was no significant difference in the expression of the Th1/Th2 cytokines IL-12, IL-10 or IFN γ between the two genotypes, nor was there a difference in the ratio of pathogen-specific IgG isotypes, which has been shown to reflect the Th1/Th2 balance [39]. These data suggest that the adaptive immune response to polymicrobial infection is not altered in the absence of OPN.

However, neutrophil numbers were observed to be increased in OPN $-/-$ mice at early times after infection, and there was an increased ratio of neutrophil elastase to iNOS in the OPN-deficient mice as compared to WT, demonstrating that the balance of neutrophils and macrophages is altered in the absence of OPN. These data support the scenario of a *lower* initial neutrophil accumulation (during the first day), followed by a *compensatory increase* at later times (days 3–7). These studies demonstrate that OPN is a key regulator of the inflammatory response in experimental endodontic infection.

The mechanism of the effect of OPN in inflammation is still unclear. OPN binds to a series of integrins through two adjacent sequences in the center of the molecule. The first of these, GRGDS binds to a series of α v-containing integrins, including α v β 3, α v β 5 and α v β 1. Immediately adjacent is the SVAYGLR (in mouse) sequence that mediates binding to the α 4 β 1 and α 9 β 1 integrins—the α 9 β 1 interaction requires cleavage of OPN at an adjacent thrombin cleavage site (reviewed in [37]). These interactions enhance migration of neutrophils in a mouse model of T-cell mediated liver disease, [40] and in a model of alcohol-induced liver injury [41], as well as in vitro [42]. The role of OPN in enhancing macrophage migration has also been repeatedly demonstrated and recently reviewed [37]. Together, these data support the hypothesis that OPN regulates both neutrophil and macrophage accumulation in vivo, particularly in infection and inflammation.

Integrins are heterodimeric transmembrane signaling proteins that can mediate both inside-out and outside-in signaling [43, 44]. Classic integrin functions include adhesion mediated by integrin binding to immobilized ligands, assembling a perimembrane adhesion/signaling complex including focal adhesion kinase (FAK) and talin [45] and mediation of cell migration, through a complex mechanism that requires formation and turnover of adhesion complexes [46], resulting from inside-out signaling. A central role of endocytic recycling of integrins in this process has recently been described [47]. In addition, integrins and their downstream signaling moieties interact with other receptors to modulate their signaling in some cases also through enhancing endocytic recycling [48, 49]. Thus, an alternative role of OPN in regulation of inflammation may be through regulation of signaling in innate immune cells through binding to the α v β 3 integrin. Since OPN is a soluble integrin ligand,

such binding could enhance endocytic recycling of integrins and associated signaling molecules, such as TLRs. This role of OPN in facilitating cell migration may be an important aspect of its function in vivo.

Finally, OPN may regulate myeloid cell phagocytosis. OPN-deficient macrophages have been shown to be deficient in mycobacterial cell killing [30] or phagocytosis [50]. In addition, OPN-deficient macrophages display reduced cytotoxicity towards tumor cells [51]. Together, these results support the idea that OPN is important for both neutrophil and macrophage migration as well as some aspects of macrophage function. Further research will be needed to describe these functions of OPN in detail.

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A Potential Mechanism for the Development of Dental Fluorosis

Megan L. Sierant and John D. Bartlett

Abstract. Currently, the mechanism behind the development of dental fluorosis remains unclear, but it is known that fluorotic enamel has higher protein content and is therefore softer than nonfluorosed enamel. Previously it was demonstrated that fluoride induces phosphorylation of the eIF2 α ribosomal component, which significantly decreases protein synthesis. This occurs during the maturation stage of development when proteins are normally removed from the hardening enamel. By combining these data with current knowledge of ameloblast function during enamel development, we can hypothesize a potential mechanism in which excess fluoride results in increased protein levels and softened enamel via decreased protease secretion during the maturation stage. Briefly, this hypothesis states that phospho-eIF2 α -mediated inhibition of protein production induced by intracellular fluoride results in decreased secretion of the enamel protease kallikrein-4 (KLK4) during the enamel maturation phase. This in turn results in decreased protein breakdown and higher protein content within the enamel matrix.

Key words. Ameloblast, Dental fluorosis, eIF2 α , Enamel, Fluoride

M.L. Sierant and J.D. Bartlett (✉)

Department of Cytokine Biology, The Forsyth Institute, 245 First Street,
Cambridge, MA 02142, USA
and

Department of Developmental Biology, Harvard School of Dental Medicine,
Boston, MA 02115, USA

e-mail: jbartlett@forsyth.org

1 Dental Fluorosis

Fluoride treatment, via either ingestion or topical methods, has been shown to significantly reduce the prevalence of dental caries in both developing and developed teeth. Incorporation of fluoride ions into the hydroxyapatite crystals of enamel increases hardness and reduces demineralization, which can delay caries formation. Excess fluoride, however, has a detrimental effect on developing enamel, resulting in opaque mottling, pitting and, in severe cases, discoloration of enamel. These areas of fluorotic enamel are undermineralized, containing increased levels of protein when compared to areas of nonfluorosed enamel [1–3]. The mechanism responsible for developing fluorotic enamel remains poorly understood.

2 Enamel Development

Enamel is produced by specialized epithelial cells called ameloblasts, which are polarized columnar cells capable of secreting large quantities of proteins. The chief function of an ameloblast is to support the growth of a single hydroxyapatite enamel rod by secreting scaffold proteins during the secretory stage, in which the rods grow in length, and then removing these proteins during the maturation stage, in which the rods thicken. There are several stages to the life cycle of an ameloblast, the early phase, called the presecretory stage, focuses on cell growth and differentiation, ensuring the cellular machinery, such as the endoplasmic reticulum, are sufficient for the next stage. In the secretory stage, ameloblasts secrete enamel scaffold proteins, which support the growing enamel crystals, and the protease matrix metalloproteinase-20 (MMP20, enamelysin), which cleaves these scaffold proteins, a step required for their proper function (reviewed in [4]). At this stage, the developing enamel is extremely soft, containing a high amount of protein, and the decussating enamel rod pattern begins to form. Additionally, in this stage, the apical end of the ameloblast forms the Tomes' process, a conical-shaped extension of the cell surface from which proteins are secreted into the enamel matrix. Ameloblasts transition into the maturation phase by shortening, retracting their Tomes' processes, and gaining either a ruffle—or smooth-ended apical cell surface. During this stage, MMP20 secretion decreases and secretion of kallikrein-4 (KLK4) increases. Like MMP20, KLK4 also cleaves the scaffold proteins in the enamel matrix but instead of being required for proper function, this facilitates their resorption by the ameloblasts. This ultimately results in a reduction of the enamel protein content from greater than 30% to less than 1% [5] and allows the enamel rods further room for growth in both width and thickness. By the end of the maturation stage, the enamel is fully hardened. In summary: ameloblasts secrete stage-specific proteases crucial for enamel rod growth and maturation as well as absorbing digested protein fragments necessary for increasing enamel hardness.

3 The Acid Hypothesis

As previously mentioned, the exact mechanism behind the development of dental fluorosis is currently unknown. However, fluoride has been shown to have no effect on the specific activity of either MMP20 or KLK4, ruling out inactivation of the enamel proteases during development as a possible mechanism [6]. During the secretory stage, the neutral pH of the enamel matrix remains stable, but in the maturation stage the pH oscillates from neutral to mildly acidic (reviewed in [7]). This results from deposition of hydroxyapatite, which releases hydrogen ions during crystallization and, in turn, decreases the pH of the enamel matrix. In addition to their secretion and absorption functions during enamel maturation mentioned above, ameloblasts also control the pH of the enamel matrix by secreting bicarbonate into the matrix and utilizing ion transporters to absorb hydrogen ions from the matrix. Previously our group has hypothesized that the presence of fluoride during the acidic phases of enamel maturation results in the formation of increased levels of highly toxic hydrogen fluoride (HF) [8]. Because HF is a weak acid and can easily diffuse through cell membranes, it diffuses down a concentration gradient into the cell. Once in the neutral cytosol of the cell, hydrogen fluoride would then dissociate into its component ions. The Henderson–Hasselbalch equation states that 25-fold more HF is present at a pH of 6.0 than at a pH of 7.4. It was demonstrated that activation of stress response genes occurred at lower fluoride doses and that fluoride-mediated inhibition of protein secretion was increased under acidic conditions when compared to neutral conditions [8]. Sharma et al. [8] hypothesized this was a result of increased intracellular fluoride levels in cells grown under acidic conditions.

4 Intracellular Stress Responses to Fluoride

Previously our group has shown that inactivation of the translational control protein eukaryotic initiation factor 2 (eIF2) via phosphorylation on its alpha subunit (eIF2 α) occurs in both cultured cells and in ameloblasts from mice exposed to fluoride [8–10]. The unfolded protein response (UPR) responds to misfolded proteins in the ER by increasing transcription of chaperones and stress response genes, and also by generally decreasing overall protein synthesis, which in turn decreases ER load. During the UPR, the PERK kinase acts to decrease protein synthesis via an inhibitory phosphorylation of eIF2 α at S51 [11]. This prevents eIF2B from hydrolyzing GTP and initiating the elongation step of protein translation. eIF2 α can also be phosphorylated by three other known kinases, each responding to different stimuli: heme-regulated inhibitor (HRI) responds to heme deprivation, arsenite exposure, heat shock and oxidative stress; protein kinase RNA-activated (PKR) in response to dsRNA resulting from viral infection; and general control nonrepressed 2 (GCN2) in response to UV exposure and nutrient limitation (reviewed in [12]). Other groups have shown activation of PERK-mediated signaling in response to fluoride in cultured osteoblasts suggesting PERK plays an important role during the development of

skeletal fluorosis [13]. 3-D culture of these cells in the presence of fluoride also resulted in increased levels of reactive oxygen species, which induced oxidative response pathways [14], and HRI has been shown to be activated by Hsp90 in response to oxidative stress [15, 16]. Whether fluoride activates PERK via ER-stress or HRI via oxidative stress or both, these data provide a strong foundation to begin investigations into the role of the eIF2 α kinases during the cellular response to fluoride.

5 Combined Theory of Dental Fluorosis

The Acid Hypothesis, described above, states that, due to pH fluctuations in the enamel matrix during the maturation phase, HF is able to diffuse down a concentration gradient into the cytosol where it dissociates into its component ions [8]. In vivo and in vitro data show fluoride exposure results in eIF2 α phosphorylation [8, 10], which is a well-characterized mechanism for halting protein synthesis [12]. qPCR on enamel organs from mice exposed to fluoride also show decreased levels of maturation stage-specific mRNA, KLK4 and amelotin (Amtn) [8]. By combining these data, we can conclude that excess intracellular fluoride results in decreased KLK4 production and secretion via phospho-eIF2 α , which accounts for the increased levels of protein seen in fluorotic enamel. Ongoing work is focused on determining which of the upstream eIF2 α kinases is involved in the intracellular response to fluoride during the acidic maturation phase as well as determining the extent, if any, of the involvement of UPR stress pathway.

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Author Index

A

Abiko, Y., 102, 176, 221, 317
Aida, J., 291, 294, 306
Akai, Y., 297, 309
Akatsuka, R., 246
Alfirano, 72
Alshwaimi, E., 383
Anada, T., 237, 246, 321, 352
Andou, K., 147
Aonuma, S., 297, 309
Aonuma, T., 125
Arahira, T., 229, 249
Arakaki, M., 327, 346
Asakawa, A., 107
Asakawa, T., 107, 114
Asami, Y., 173

B

Bartlett, J.D., 367, 396, 408
Batbayar, B., 46
Bazar, A., 46
Beighton, D., 195

C

Chen, G., 249
Chen, Z.-X., 255
Chiba, M., 110, 112
Cho, J., 268
Chosa, N., 107, 114
Chuanbin, G., 38

D

Daimaruya, T., 355
Deguchi, T., 355

Dilinuer, M., 252, 285
Domon-Tawaraya, H., 178, 192
Duyck, J., 349

E

Ebihara, Y., 265
Echigo, S., 140
Egusa, H., 9
Endo, N., 119, 122
Endo, T., 257, 271
Endo, Y., 95, 104, 235

F

Finger, W.J., 260
Fujii, T., 125
Fujita, K., 186
Fujiyama, K., 355
Fukumoto, E., 125
Fukumoto, S., 14, 128, 178, 183, 327, 346
Fukunaga, T., 102
Funahashi, R., 300
Funaki, Y., 156, 362
Furuya, J., 145

G

Goto, T., 352

H

Hagiwara, M., 161
Hakami, Z.W., 125
Hamada, M., 317
Hamada, T., 252, 285
Hanaoka, K., 297, 309

Han, X., 173, 373
 Harada-Oikawa, R., 198
 Harako, J., 223, 297, 309
 Hasegawa, A., 181, 221
 Hasegawa, T., 107, 114
 Hashimoto, K., 221
 Hasturk, H., 376, 387
 Hata, S., 134
 Hattori, Y., 83, 97, 312, 315
 Hayashi, E., 150
 Hayashi, H., 110, 112,
 131, 159
 Hidetoshi, S., 303
 Hinokio, Y., 212
 Hiratsuka, K., 102
 Hoffman, M.P., 3
 Honda, Y., 237
 Hong, G., 252, 285
 Hong, J., 383, 393
 Hongwei, L., 38
 Hoshikawa, Y., 181
 Hoshino, T., 257, 260
 Hosokawa, R., 223, 297,
 300, 309

I

Igarashi, K., 192
 Igari, Y., 83
 Ikai, H., 86, 89, 150, 240
 Ikawa, K., 303
 Ikawa, M., 117, 128, 303
 Ikawa, S., 92
 Ikeuchi, T., 183
 Imai, Y., 312, 315
 Ina, Y., 147
 Inagaki, R., 240, 263, 268
 Ishihata, H., 22, 232, 246
 Ishii, K., 362
 Ishikawa, T., 204, 206
 Ishimoto, T., 55
 Ishisaki, A., 114
 Ishiyama, K., 89
 Isizaki, A., 107
 Itabashi, S., 312, 315
 Itagaki, K., 297, 309
 Ito, E., 223, 300
 Ito, H., 100
 Ito, K., 291, 294
 Iwama, N., 22, 232
 Iwamatsu, M., 122
 Iwamatsu-Kobayashi, Y., 119, 122

Iwamoto, T., 327, 346
 Iwasawa, A., 86

J

Jin, J.-O., 393

K

Kajiya, M., 383, 393
 Kanehira, M., 260
 Kanetaka, H., 131, 167
 Kanno, T., 86, 89, 150, 240
 Kano, M., 131, 167
 Kantarci, A., 376, 387
 Kanzaki, H., 173
 Karita, K., 128
 Kasahara, S., 265
 Kato, A., 140
 Kato, K., 186
 Katoh, H., 265
 Kawai, T., 173, 373, 383, 393
 Kawamura, H., 235, 243
 Kawanami, M., 164
 Kawano, T., 22, 232
 Kawashima, J., 189
 Kawata, V.K., 92
 Keiichi, S., 285
 Kikuchi, K., 265
 Kikuchi, M., 66, 122, 268, 278
 Kikuchi, T., 341
 Kikuchi, Y., 362
 Kim, S., 29
 Kimura, K., 125, 297, 309
 Kimura, S., 145, 198, 204, 206, 283
 Kinbara, M., 95
 Kindaichi, J., 119
 Kindaichi, K., 119
 Kitaura, H., 125
 Kiyama, T., 104, 235
 Kodama, Y., 204
 Kohno, M., 86, 89, 150, 240
 Koketsu, M., 161
 Komagata, M., 297, 309
 Komatsu, H., 117, 128
 Komatsu, M., 119, 122, 257, 260, 271,
 273, 341
 Komine, Y., 83, 97
 Kondo, T., 181
 Kondoh, D., 341
 Koseki, T., 153, 212, 223, 297, 300, 306, 309
 Koyama, S., 209, 362

Koyano, K., 137, 229
Kudo, M., 268
Kudo, T., 131, 167
Kumamoto, H., 134
Kurihara, J., 243
Kuriyagawa, T., 246

L

Li, W., 31

M

Matsubara, T., 255
Matsumoto, S., 186
Matsumura, K., 246
Matsushita, K., 161, 317
Matsushita, Y., 137, 229
Matsuura, T., 92
Matsuyama, J., 221
Mayanagi, G., 192, 218, 334
Miki, Y., 134
Min, C., 393
Mineta, S., 72
Miura, E., 257
Miura, S., 268
Miyai, R., 110, 112
Miyamoto, R., 327
Miyoshi, Y., 312, 315
Mizuno, K., 131
Mizuta, K., 359
Mokudai, T., 240
Morimoto, S., 237
Mori, S., 153
Morita, Y., 137
Motohide, I., 303
Murakami, T., 102, 312, 315

N

Naert, I., 349
Nagai, Y., 95
Nakagaki, H., 186
Nakajo, K., 178, 189, 192, 195, 209
Nakamura, K., 86, 89, 150, 240
Nakamura, M., 140, 147
Nakamura, T., 14, 183, 327, 346
Nakano, T., 55
Naruse, M., 327
Narushima, T., 72, 243, 352
Nemoto, E., 104, 143
Nemoto, T.K., 206

Niinomi, M., 352
Ninomiya, M., 161
Nishihara, D., 119, 122
Nishihira, T., 317
Nishitani, M., 317
Nishiwaki, N., 243
Niwano, Y., 86, 89, 150, 240
Noguchi, Y., 291
Noji, M., 246
Nomura, T., 145

O

Ogawa, T., 349
Ohara-Nemoto, Y., 198, 206
Ohba, T., 300
Ohi, T., 312, 315
Oizumi, T., 235
Okada, S., 235
Ok, P., 383, 393
Okuya, F., 297, 309
Ono, K., 134
Ono, M., 153, 346
Osaka, K., 291, 294
Otake-Asakawa, A., 198
Ouhara, K., 393
Ou, Y., 186

P

Pagonis, T.C., 383, 393

Q

Qu, X., 117

R

Ren, L.-M., 255
Rittling, S.R., 402
Ryu, Y., 131

S

Saito, K., 153
Saito, R., 134
Sakai, Y., 355
Sakakibara, S., 186
Sakashita, R., 176, 317
Sakuma, Y., 201, 209
Sakurai, Y., 215
Sano, Y., 167

Sasaki, H., 362, 383
 Sasaki, K., 86, 89, 104, 147, 150, 201,
 209, 237, 240, 246, 252, 268,
 285, 349, 352, 362
 Sasaki, M., 145, 204, 206, 283
 Sasano, H., 134
 Sasano, Y., 140, 147
 Sasazaki, H., 257, 271, 273
 Satake, M., 92
 Sato, H., 271, 273
 Sato, M., 83, 164
 Sato, N., 352
 Sato, T., 176, 181, 186, 209, 221, 317
 Seiryu, M., 355
 Shimamura, A., 215
 Shimauchi, H., 22, 117, 143, 189, 221,
 232, 275, 303
 Shimizu, Y., 131, 167
 Shimomura, M., 22, 232
 Shimoyama, Y., 145, 204, 206
 Shiraishi, N., 352
 Shirato, M., 86, 150
 Shiwaku, Y., 237
 Sierant, M.L., 396, 408
 Silva, M.J.B., 383
 Sonobe, H., 297, 309
 Stashenko, P., 383, 393
 Sugawara, S., 95, 104, 235
 Sugiyama, M., 306
 Sunghwa, F., 161
 Suzuki, J., 223
 Suzuki, M., 173
 Suzuki, O., 237, 246, 321, 352
 Suzuki, T., 145
 Suzuki, Y., 352

T

Tada, H., 143
 Taira, M., 283
 Takada, H., 183
 Takada, Y., 66, 240, 278
 Takahashi, H., 156
 Takahashi, M., 66, 278, 300
 Takahashi, N., 43, 176, 178, 181, 189,
 192, 195, 201, 209, 212, 218,
 221, 334
 Takano, H., 104
 Takano-Yamamoto, T., 95, 102, 125, 355
 Takao, Y., 255
 Takayama, T., 100
 Takeda, Y., 161
 Takeshita, N., 102

Takeuchi, K., 291, 294
 Takeuchi, Y., 201, 209, 221
 Taku, S., 303
 Tamahara, T., 223, 297, 309
 Tamate, S., 243
 Tamazawa, K., 275, 280
 Tamazawa, Y., 275, 280
 Tamura, K., 186, 297, 309
 Tamura, M., 164
 Tanaka, M., 107, 114, 198
 Tanaka, Y., 83
 Tanda, N., 212, 223
 Tanigawa, N., 161
 Tao, M., 317
 Tao, X., 38
 Taubman, M., 173
 Taubman, M.A., 373
 Taura, K., 223
 Toda, T., 159
 Todo, M., 100, 137, 229, 249
 Tsuboi, A., 156, 312, 315
 Tsuchiya, M., 104
 Tsuchiya, S., 104
 Tsumori, H., 215
 Tu, R., 352

U

Ueda, K., 72, 243, 352
 Uyama, M., 164
 Uzuka, R., 352

V

Van Dyke, T.E., 376, 387

W

Wakaguri, S., 291, 294
 Wakamori, M., 341
 Wang, W., 252, 285
 Wang, W.-X., 255
 Washio, J., 178, 189, 192, 201, 212,
 218, 334
 Watanabe, M., 104, 156, 312, 315
 White, R.R., 383, 393
 Wirianski, A., 83

Y

Yamada, A., 327, 346
 Yamada, Y., 14, 240
 Yamaguchi, K., 235

Yamaguchi, S., 156
Yamakami, K., 215
Yamaki, K., 221
Yamamoto, M., 362
Yamashiro, T., 102
Yamazaki, H., 362
Yoda, M., 263, 268
Yokota, S., 243
Yokoyama, M., 362
Yong, J., 38

Yoshida, E., 223
Yoshida, T., 341
Yoshimura, Y., 114
Yuki, I., 300

Z

Zhang, X., 349
Zhang, Y., 131, 167
Zhou, J., 31

Keyword Index

A

Abiotrophia/Granulicatella, 206
Acetone, 212
Acid production, 189
Acrylic denture base resin, 252
Acrylic resins, 145, 201, 209
Actinomyces naeslundii, 189
Adenylyl cyclase, 359
Adherence, 145, 204
Adult rats, 140
Airway smooth muscle, 359
AlamarBlue®, 201
Allogenic transplantation, 147
Alveolar ridge augmentation, 376, 387
Ameloblast, 367, 396, 408
Ameloblastin, 327
Ameloblastoma, 327
AMP-activated protein kinase (AMPK), 131
Animal experiment, 243
Animal model, 387
Anodic spark oxidation, 255
Apatite orientation, 56
Apoptosis, 125, 373
Argon, 257
Aryl hydrocarbon receptor, 134
Asthma, 359
ASTM F75, 72
ATDC5, 102
Atopic dermatitis, 273
Autotransplant, 232

B

Backscattered electron image, 186
Bacteria, 218, 280
Bacterial adhesion, 201
Bacterial detection, 209

Bacterial identification, 209
Bactericidal effect, 86, 150
Bacteriostatic biomaterials, 66
 β -stabilizing element, 66
Bicarbonate, 189
Bifidobacteria, 195
Biodegradation, 321
Biofilm, 86, 176, 215, 280
Biofilm removing capacity, 186
Biomaterials, 192, 201, 278
Bisphosphonate, 235
Blood flow, 117
B lymphocytes, 373
BMP-2, 143
Bone
 formation, 321
 metabolism dental implant, 362
 model, 137
 quality, 56, 229
 regeneration, 110
 resorption, 383
Bone morphogenetic protein (BMP), 131
Bortezomib, 164
Bronchial fluids, 181

C

CAD/CAM, 265
Cadherin, 367
Calcium phosphate, 243
Calcium phosphate coated implant, 352
Calculus, 300
Candida albicans, 145
Capsaicin, 140
Care-provider, 306
Caries, 215
Cartilage, 346

Cell differentiation, 164
Cell-ELISA, 162
Cell migration, 396
Cell signaling, 396
Cellular morphology, 22
CE-TOFMS, 218
c-Fos, 355
Channel, 341
Chewing-cycle trajectory, 83
 χ -phase, 72
Chondrogenic induction, 119
Chronic periradicular periodontitis, 393
Clinical application, 246
Clinical attachment loss, 312
Clinical performance, 271
Clodronate, 235
Cluster analysis, 83
Co-Cr alloy, 72
Cognitive function, 317
CO₂ laser, 355
Collaboration, 298
Collagen scaffold, 249
Columnar organization, 159
Community dentistry, 310
Composites, 100
Compression force, 112
Compressive modulus, 249
Contact dermatitis, 273
Co-precipitation, 237
Corrosion resistance, 66, 240
Cross-reaction, 95
Crown, 265
CT images, 265
Curing depth, 260

D

Deciduous tooth, 114
Dental alloy, 278
Dental CAD/CAM, 263
The Double-Degree program, 43
Dental education in Mongolia, 47
Dental epithelium, 327
Dental fluorosis, 408
Dental implant, 137, 229, 243
Dental metals, 240
The Dental School We Aspire, 29
Dental unit, 280
Dentin bond strength, 257
Dentistry, 9, 38
Denture plaque, 201
Dextranase, 215
Diagnosis, 223
Differentiation, 93

Digital image correlation, 137
Dioxin, 134
1,3-Diphenylisobenzofuran (DPIBF), 89
Divalent cations, 178
Dopamine, 359
Dopaminergic system, 156
Dorsomorphin, 131
Dullard, 168

E

Edentulous maxilla, 229
Education, 38
eIF2 α , 408
Elderly, 176, 181, 317
Electrogastrography, 97
Electron beam melting (EBM), 56
Electron spin resonance, 89
Enamel, 367, 408
Enamel development, 396
Endogenous acid production, 195
Epigallocatechin gallate, 153
Epiprofin, 14
E-selectin, 162
Etidronate, 235
External joint type, 263

F

Facility-user, 306
Fatigue, 105
FEA. *See* Finite element analysis (FEA)
Fibroblast growth factor-2, 107
Fibronectin binding activity, 204
fimA, 176
Fingerpad, 128
Finite element analysis (FEA), 229, 263
Flavonoid, 162
Flow rate, 223
Fluorescent probe, 89
Fluoride, 178, 189, 237, 408
Formaldehyde, 303
Fracture, 255
Fracture toughness, 268

G

Gap junction, 346
Gastric motility, 97
Gender difference, 294
Gene expression, 112
Gingival fibroblast, 9
The Global 30 program, 43
Glucose metabolism disorder, 312

H

Health promotion, 317
 Honeycomb-patterned porous polymer film, 22, 232
 Hospital, 298
 Hospitalized patients, 298, 310
 hTERT, 114
 Human lip, 128
 Human wisdom tooth germ, 119
 Hydrogen peroxide (H_2O_2), 86, 150, 240
 Hydrolysis, 237
 Hydrolysis degradation, 100
 Hydroxyapatite film, 246
 Hydroxyl radical, 86, 150, 240
 Hypersensitivity, 95

I

Identification, 206
 IL-6, 105
 IL-12, 125
 IL-18, 125
 Immediate dental implant, 376
 Immediate implant placement, 387
 Immediate loading, 376, 387
 Immobilize, 114
 Immunocytochemistry, 122
 Immunofluorescent confocal microscopy, 393
 Implant, 263
 Induced pluripotent stem (iPS) cells, 9
 Infected root canal, 221
 Infection, 265, 402
 Infection control, 280
 Inflammation, 95
 Inflammatory cytokines, 283
 Inhibition of acid production, 178
 Innate immunity, 183
 Integrin, 402
 Interdisciplinary, 43
 Interface, 352
 Internal joint type, 263
 International, 43
 Ion-sensitive field-effect transistor pH electrode, 192
 iPS cells. *See* Induced pluripotent stem (iPS) cells
 Isolation rate, 122

K

Keratin 5, 3
 Knoop hardness (KHN), 260

L

Laser Doppler, 117
 LED, 150
 Liaison office, 310
 Lichen planus, 273
 Lipopolysaccharide, 95
 Low-magnitude and high-frequency loading, 350

M

Machinability, 66
 Macrophage, 283, 402
 Mandible, 56
 Mandibular condylar cartilage, 102
 MAPK, 183
 Marginal adaptation, 271
 Mastication, 97, 105
 Maternal transmission, 198
 Matrix metalloproteinase-20 (MMP20), 367
 Maxillary obturator-prostheses, 209
 Mechanical loading, 362
 Mechanical properties, 66, 100
 Medullary dorsal horn, 355
 Mesenchymal stem cells, 22, 119, 122, 249
 Metabolism, 218
 Metabolome analysis, 334
 Metabolomics, 31, 218, 334
 Metabonomics, 31
 Metal allergy, 95, 273
 Microbeam X-ray diffraction, 56
 Microbiota, 221, 334
 Micro-CT, 147
 Microdialysis, 156
 Micro flora, 176
 Micro-space, 110
 Microstructure, 255
 MMP20. *See* Matrix metalloproteinase-20 (MMP20)
 Motility, 367
 Movement, 367
 mRNA, 393
 Mutanase, 215
 Mutans streptococci, 189, 195, 198

N

N-cadherin, 396
 Needs, 306
 Neuron, 159
 Neuronal differentiation, 168
 NF- κ B, 183
 Nitric oxide, 125
 Nociception, 140
 NOD2, 183

Non-mutans streptococci, 198
 Non-toxic gas, 275
 Nursing home, 306

O

Octacalcium phosphate, 237, 321
 OPG. *See* Osteoprotegerin (OPG)
 Oral bacteria, 181
 Oral biofilm, 334
 Oral care, 310
 Oral disease, 31
 Oral health care, 298
 Oral health-related quality of life, 315
 Oral implant, 350
 Oral irrigator, 186
 Oral mucosal epithelial cells, 204
 Oral plaque biofilm, 218
 Oral science, 31
 Osseointegration, 352
 Osteoblastic markers, 93
 Osteoblasts, 93, 110, 112, 134, 321
 Osteoclastogenesis, 173
 Osteoclasts, 125, 134, 321, 383
 Osteogenesis, 134
 Osteogenic induction, 119
 Osteonecrosis, 235
 Osteopontin, 402
 Osteoprotegerin (OPG), 393
 Oxygen, 275
 Oxygen inhibition, 257

P

p63, 93
 Pannexin, 346
 Parasite, 192
 Particle size distribution, 100
 The pattern of remaining
 teeth, 315
 PC12, 168
 PCR, 221
 Peel bond strength, 285
 Peri-implant bone, 350
 Periodontal bone loss, 373
 Periodontal disease, 312
 Periodontal ligament, 122
 Periodontal ligament cells, 22, 107, 112, 114, 232
 Periodontal pocket, 300
 Periodontal probe, 300
 Periodontal regenerative
 therapy, 232
 Periodontitis, 173
 Periradicular periodontitis, 383
 Permanent tooth, 107

PET scanner. *See* Positron emission
 tomography (PET) scanner

pH, 192
 Phagocytosis, 283
 pHin, 195
 Phosphatase, 168
 Phosphate, 143
 Photolysis, 86
 Physiochemical interaction, 192
 π -phase, 72
 Plasma, 275
Porphyromonas gingivalis, 176
 Positron emission tomography, 156
 Positron emission tomography (PET)
 scanner, 362
 Powder jet deposition, 246
 Precipitation, 72
 Principal component analysis, 83
 Probing force, 300
 Progenitor cell, 3
 Proliferation, 93
 Protein adsorption, 237
 Pulp, 117
 Pulse-field gel electrophoresis, 198
 Pustulosis palmaris et plantaris, 273

Q

Quality of life, 306
 Quantitative evaluation, 186

R

Radiofrequency magnetron sputtering system, 352
 RANKL. *See* Receptor activator of nuclear
 factor-kappa B ligand (RANKL)
 Rats, 147, 156, 355
 Rat tibia, 350
 Receptive field, 159
 Receptor activator of nuclear factor-kappa B
 ligand (RANKL), 373, 383, 393
 Regeneration, 3
 Regenerative medicine, 249
 Remodeling, 112
 Replica, 271
 Reprogramming, 9
 Resilient denture liner, 285
 Resin composite, 260
 Resting saliva, 223
 Restriction fragment length
 polymorphism assay, 206
 RF magnetron sputtering, 243
 Root canal, 303
 Root canal therapy, 221
 Root development, 147

Root surface, 300

Rose bengal, 89

S

Salivary gland, 3

Scaffold, 110

School of Dentistry, 47

Self-etch bonding system, 271

Self-management, 317

Self-organization, 22

Self-rated oral health, 294

SEM, 271

Sericin powder, 252

Sham feeding, 97

Short-time effect, 178

Silent aspiration, 181

Singlet oxygen, 89

Sjögren's syndrome, 153

Skeletal muscle, 105

Socioeconomic status, 291, 294

Somatosensory cortex, 159

Species-specific PCR assay, 206

Spore, 275

sRANKL, 173

16S rRNA, 181, 221

Stem cell, 3

Sterilization, 275

Stimulated saliva, 223

Stomatology, 38

Strain distribution, 137

Streptococci, 215

Streptococcus anginosus, 204

Streptococcus mutans, 178

STRO-1, 119, 122

Stromal cell-derived factor 1, 107

Sub-micron particles, 283

Supernumerary teeth, 14

Supra-organism, 334

Surface status, 145

Surface treatment, 285

Surface wettability, 252

Synthetic bone graft, 376

T

TACE. *See* TNF- α converting enzyme (TACE)

Tarnish resistance, 278

Taste bud, 341

T cells, 383

Temporal correlation, 159

Ten-m/Odz3, 102

Thermal energy, 150

Thymidine glycol, 153

Ti-Ag alloy, 278

Ti-29Nb-13Ta-4.6Zr alloys (TNTZ), 352

Tissue blood oxygenation, 128

Tissue engineering, 9

Titanium, 255, 283

TNF- α , 125, 173

TNF- α converting enzyme (TACE), 173

TNTZ. *See* Ti-29Nb-13Ta-4.6Zr alloys (TNTZ)

Toll-like receptor, 373

Tooth, 117, 147

development, 14, 327

loss, 291, 312

morphogenesis, 14

movement, 355

Tooth-biomaterial interface, 246

Topology, 22

Transient receptor potential (TRP), 341

Transmission electron microscopy, 140

Trigeminal nerve, 140

TRP. *See* Transient receptor potential (TRP)

Type 1 diabetes mellitus, 212

Type 2 diabetes mellitus, 212

U

Ubiquitin-proteasome pathway, 164

V

Veneer porcelain, 268

Viability, 195

Vickers indentation, 268

Vitamin A, 383

Vitamin D₃, 183

W

Water contamination, 280

Water supply pipe, 280

Whole body vibration, 350

Whole-field measurement, 137

Working environment, 303

X

Xerostomia, 223

Y

Yttria-stabilized tetragonal zirconia
(Y-TZP), 268

Z

Zirconia all-ceramic, 268

Zoledronate, 235